Presented for the Degree of M.D., University of Edinburgh:

by

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(a) THESIS - Recent Advances in the Biology and Treatment of Certain Fungus Diseases of the Skin.

(b) Seven published papers on dermatological problems:

(1) The calcium content of the blood serum in skin diseases. G.H. Percival and C.P. Stewart. Brit. J. Derm. Syph. 1927, 39, 144.

(2) Some observations on a condition of chronic erythema of the legs. G.H. Percival and C.P. Stewart. Ibid. 39, 115.

(3) Melanogenesis: a review. G.H. Percival and C.P. Stewart. Ed. Med. J. Sept. 1930.

(4) Observations on the aetiology of erythema exudativum multiforme. G.H. Percival and H.J. Gibson. Brit. J. Derm. Syph. 1931, 43, 329.

(5) A study of the skin vessels in some forms of inflammation of the skin. G.H. Percival and C.M. Scott. J. Pharm. Exper. Therap. 1931, 41, 147.

(6) On the sulphydryl-containing constituent of the epidermis and its relationship to melanogenesis and keratinization. G.H. Percival and C.P. Stewart. Brit. J. Derm. Syph. 1930, 42, 215.

(7) Experimental Observations on Dermatitis due to Dyed Fur. G.H.Percival. Lancet. 1931, p.417.

Contents.

INTRODUCTION.

Since Gruby's discovery in 1841 that Favus was due to an infection by a vegetable parasite mycology has assumed a place of increasing importance in the study of diseases of the skin. During the succeeding ninety years the progress of this branch of dermatology has been slow, and from time to time has been impeded by useless controversy. Even now the subject is far from exhausted, and, coindicent with the trend of investigation in other branches of scientific medicine, is passing from the purely morphorphological phase into the realms of biology and biochemistry.

A period of transition in any system, whether of cells or ideas, invariably renders such a system liable to misconstruction and imperfect development. It is a period when a review and stabilisation of what has gone before is an invaluable asset, forming

a/

a firm basis for the further judicious advancement and growth of the system.

Such an historical and critical review of mycology in its relation to dermatology has been attempted in this thesis. It comprises literary research, and a record of the results of the author's personal investigations into certain of the problems which the subject presents.

The number and incidence of definitely established mycotic diseases of the skin has greatly increased within recent years. This increase may be due to a greater prevalence or infectivity of the causative fungi, to altered social and environmental conditions leading to greater opportunities for infection, or to the concentration of attention on this type of disease with the consequent recognition of cases which would previously have been placed in another category. Coincident with the more frequent clinical recognition of mycotic eruptions pathogenic properties have been attributed, rightly or wrongly, to an ever increasing variety of fungi, and in addition attention has been directed towards the biological/

biological aspect of the subject. As a result the literature has increased enormously, so that it becomes difficult to separate the essential advances which have been made from the mass of botanical and clinical detail with which they are surrounded. Many of the recent publications deal only with one or two cases, and the significance of the deductions made therefrom can only be assessed when these are correlated with the findings in similar conditions; otherwise undue emphasis may be laid on relatively unimportant details and incomplete observations. Moreover the classification of newly described mycotic infections as recognisable clinical entities is only possible from the survey of a large number of cases.

I. THE CLASSIFICATION OF PATHOGENIC HYPHOMYCETES.

Cultural Considerations.

Many superficial skin lesions have been attributed to an infection by species of fungi (Eumycetes). In a certain proportion of these the fungus in question has been shown experimentally to be the cause of the particular skin lesion. In the

the remainder the claim for such an origin is based only on the microscopical or cultural demonstration of a fungus in the lesions. The isolation and identification of a species which is known to be pathogenic from a skin lesion is strong evidence that the organism is responsible for the disease, and is not a commensal saprophyte. The evidence of causal relationship is strengthened when the lesion conforms in its clinical appearance and course to that resulting from a definitely proved infection with a similar species.

The microscopical appearance presented by a fungus as it occurs in material obtained from a lesion may be sufficient for the recognition of the family to which the fungus belongs. In the case of fungus-infected hair even the genus may be identified. Microscopical examination may therefore suffice to demonstrate whether the fungus elements present belong to a known pathogenic variety. To obtain this information with greater certainty cultural differentiation is necessary. Cultivation is essential in cases where doubtful fungus elements, which may belong to a saprophytic species or actually/

actually be due to optical artefacts, are seen in microscopic preparations made from the lesions.

The more precise differentiation of fungi by cultural methods has shown that certain species are generally associated with certain types of lesion, and also that two clinically similar lesions may be produced by different species of fungi. The course and severity of the disease may bear a close relationship to the species of the causative fungus, while on the other hand closely related species may produce lesions which differ in these respects.

The broad differentiation of pathogenic fungi into Orders, Families, Species and Genera is based on the type of reproductive organs which they develop in culture. From the botanical standpoint it is possible to classify fungi on this uniform standard. When, however, the anatomical position of the fungus in the lesion is considered, it becomes obvious that purely botanical criteria form an unsuitable basis for a classification which can be readily correlated with the pathology of mycotic diseases. On the other hand, an anatomical classification/

classification is unsuitable since morphologically different organisms may cause the same anatomical lesion. Langeron (1) points out that pathogenic fungi with widely differing saprophytic characteristics tend to converge in their morphological characteristics when acting as pathogenic agents. A parasitic existence would seem therefore to alter their vegatative characters while the hereditary characters remain unchanged. In some cases the morphological classification is supplemented by fermentation reactions.

Cultivation is the most important, if not the only procedure available for the classification of fungi, and to obtain uniformity of classification a standard method is necessary. The variability in quality of the ingredients of suitable media is the main obstacle to the realisation of such a standard, for the essential substance in these media is peptone which is a chemically impure compound. The problem at present is the selection of ingredients which will reproduce the distinctive characters of a fungus, only those ingredients which can be duplicated internationally being utilised. The aim of cultivation is the elaboration/

elaboration of morphological characteristics which are distinctive for each species, and luxuriance of growth is not an essential factor.

Most work has been directed towards the standardisation of cultures of the Trichophytoneae (Castellani and Chalmers)[2] originally classified and described by Sabouraud[3], and much interesting information has been obtained from this study. The object has been to devise an easily obtainable medium which will bring out the differentiating features as described by Sabouraud. Weideman and McMillan (4) (1921) showed that the success of a culture medium depended on the type of peptone used, the carbohydrate factor being of secondary importance. Adjustment of the pH of an unsatisfactory brand of peptone to that of the original Chassaing product employed by Sabouraud is not sufficient to render the former suitable. Hodges (5) confirmed these results in his investigations on M. equinum, M. lanosum, T. interdigitale, and M. felineum. He found that dextrose is the most important of the sugars for developing the growth of these fungi and maintaining their characters, crude and pure dextrose being equally efficient; the crude/

crude maltose acted in virtue of its dextrose content, and pure maltose was unsuitable. The make of peptone exerted a marked influence on the gross cultural characteristics of the fungi. The amino-acid content of the peptone is apparently more important than the pH, although a high initial pH is to be avoided. The same make of peptone may vary in pH, but the resulting differences in the cultures are slight when the medium contains dextrose. When a pure peptone conservation medium is used, however, slight differences in the pH of the peptone cause definite variations in the growth obtained. Hodges recommends either -

(1) Sabouraud's original formula:

Maltose or glucose (Chanut brand)	40 gm.
Powdered peptone (Chassaing	10 gm.
Gelatin	18 gm.
Water	1000 gm.

(2) American dextrose and Chassaing peptone

(3) American dextrose and Fairchild's peptone.

He gives the following formula, to be made up according to Sabouraud's method:

Peptone (Fairchild)	10 gm.
Dextrose (American granular)	40 gm.
Agar	18 gm.
Water	1000 gm.

The resulting pH is 5.

A/

A change in the chemical composition of a medium will affect the growth of different species differently, and that which is satisfactory for some will be unsatisfactory for others, so that if a new culture medium is introduced it must be tested on all species of fungi before its value can be assessed. Many substitutes for the original Sabouraud medium have been suggested in which the peptone and dextrose have been replaced by other compounds, but so far no really satisfactory formula has been devised which can supercede those given above. Weideman and Spring (6) state that "after study of and acquaintance with known fungus species on a given medium, it is possible for any individual worker to identify "unknowns" as grown on that medium; in short numerous formulas could doubtless be constructed and classed as satisfactory in the hands of widely separated workers".

From the clinical standpoint a minute classification is not always important, the main point being the recognition of pathogenic fungi in a lesion, a fact which can usually be ascertained in the commoner diseases by microscopical examination of/

of material from the lesion. The isolation and classification of the fungus is in most cases a corroborative measure, but it is an essential procedure when a new type of fungus is met with which hitherto has not been regarded as pathogenic. In such a case the only proof of its pathogenicity is that it can be made to reproduce the disease experimentally and be recovered from the lesions thus produced. It is perhaps in regard to the classification of newly discovered fungi that the necessity for standard methods of classification becomes most urgent; in the absence of means for rigid identification there is the risk of erroneously creating new genera and species out of known varieties which have changed their characters as a result of the medium employed in their isolation.

The classification of hyphae producing fungi proposed by Castellani is given below, supplemented by Sabouraud's morphological standards. In this classification anatomical factors are taken into account, and in addition certain fermentative properties of the monilias have been used as an aid to their identification. The latter procedure has been criticised/

criticised by Ashford who maintains that the fermentative reactions exhibited by this genus are inconstant.

Ascomycetes.

Reproduction takes place by means of Ascospores, and mycelium when present is septate.

Order Saccharomycetales - Families and genera are "yeast-like fungi."

"Monilia"

Order Aspergillales

Family Gymnoascaceae. Reproduction by mycelial or conidial spores.

Tribe Gymnoasceae.
Ascomycetes type.
Asci present.

Tribe Trichophytoneae.
Fungi imperfectitype.
Asci absent, asexual reproduction by conidia and arthrospores.

G. microsporum.
G. trichophyton.
G. ectotrichophyton.
G. neotrichophyton.
G. achorion.
G. epidermophyton.
G. endodermophyton.

Family Aspergillaceae

Moulds

The tribe Trichophytoneae differs from the yeasts in that abundant mycelial filaments are present, while free budding cells are absent. Morphologically the genera which it comprises possess well defined fructifications or specially shaped conidia.

Strictly speaking the tribe Trichophytoneae should not take its place with the ascomycetes since its members do not produce asci. Nevertheless on account of the character of its mycelium and its mode of reproduction which resembles the subsidiary methods of reproduction of other members of the ascomycete division, it is included with them. The various genera of the tribe can be further differentiated as follows:-

A. In lesions only mycelial filaments and no spores present.

(a). Conidia sessile - genus Microsporum.

(b). Conidia on short stalks.
Attack hairs or hair follicles.

(1) Grows entirely in the hair and filaments and spores cannot be found outside it; not pyogenic, except more rarely; of human origin - genus Trichophyton.

(2) /

(2) Grows in and on the surface of the hair; is often pyogenic and of animal origin - genus Ectotrichophyton.

(3) Grows mainly in the hair, but a few mycelial filaments and spores can be found outside the hair; not pyogenic; of human origin - genus Neotrichophyton.

(c) In cultures fusiform bodies present in the form of swollen claviform ends of filaments, yellow favic scutula present in lesions - genus Achorion.

B. In lesions mycelial filaments and spores present.

In cultures no conidial-bearing hyphae found; do not attack hairs or hair follicles, but grow in the superficial or deep strata of the epidermis.

(a) Pluriseptate spindles present in cultures; grow in the superficial strata of the epidermis, do not attack hairs; cultures not faviform - genus Epidermophyton.

(b) /

(b) Pluriseptate spindles unknown in cultures; grow between the superficial and deep layers of the epidermis; cultures faviform - genus Endodermophyton.

Genus Microsporum.

A. Human origin.

Species: M. Audouini - common form.

M. Velveticum
M. Umbonatum } rare forms.
M. Tardum

B. Animal Origin.

M. Canis vel lanosum - common form.

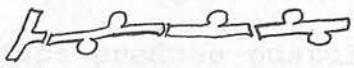
M. Felineum
M. Fulvum.
M. Villosum } rare forms.
M. Pubescens
M. Tomentosum

Morphologically the Microsporums resemble Trichophytons, but there are minor differences.

(1) The individual cells of the hyphae may be racquet shaped.

(2)/

(2) Simple external spores are found.



(3) Fusiform organs are present (These develop slowly in human types, very rapidly in animal types).

Microsporon Audouini differs from the animal species by its slower growth, the absence of pleomorphism, and the slow development of fuseau.

Genus Trichophyton.

From the anatomical standpoint there are three types of Trichophyton.

(1) Endothrix, in which the fungus elements are contained entirely within the hair.

(2) Neo-endothrix, in which the fungus elements are less strictly limited to the interior of the hair and which cause inflammatory lesions of the scalp and beard region.

(3) Ectothrix, in which the fungus elements are situated within and around the hairs. There are two/

two groups of Trichophyton Ectothrix, the microspore type or microids and the megalosporons. The members of both groups produce pustular lesions and kerions and are of animal origin.

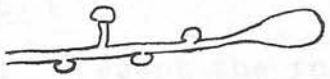
Endothrix	Endothrix pure -	T. crateriforme. T. acuminatum. T. violaceum. 12 rarely met with species
	Neo-endothrix -	T. cerebriforme. T. plicatile.
Ectothrix	Microids	Gypseum type (6 species) Niveum type (2 species)
	Megalosporons	Wooly colonies (3 species) Faviform colonies (3 species)

Mycological Characteristics of Trichophytons.

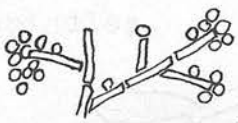
(1) Endothrix group.

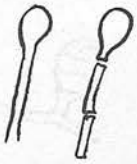
T. acuminatum.

Aerial filaments with lateral spores are the typical forms of reproduction of trichophytons. The spores are sessile or placed on short sterigma situated laterally on a thin hypha which may be laterally or terminally placed with regard to the parent mycelium.



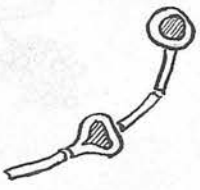
T. crateriforme. The spores are bunched.





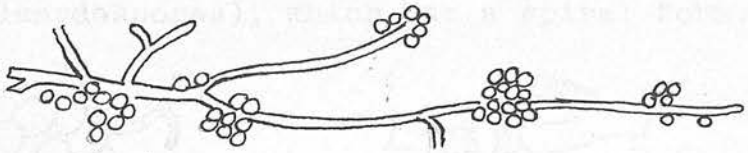
The hyphae have a terminal bulge and are either non septate, septate, or spore bearing. In spore bearing hyphae the protoplasm may not take on the stain in areas on which spores are growing.

T. violaceum. This species does not ordinarily possess lateral spores, but has intercalary chlamydospores.



Neo-endothrix group.

The species of this group are very similar to crateriforme trichophytons and show absorption of protoplasm in dry cultures.



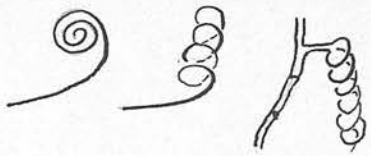
Ectothrix group.

Microide types present the following characteristics:

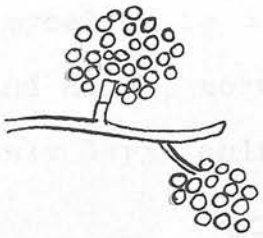
T. gypseum.

(1) Terminal chlamydospores forming septate spindles.

(2) Spiral filaments.



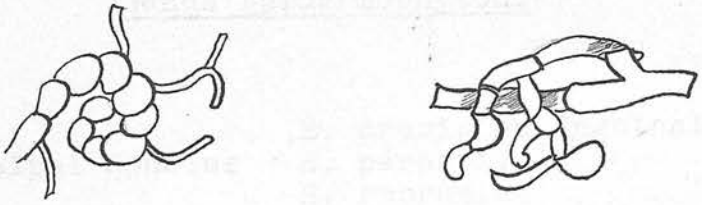
(3) Spore bunches.



It/

It may require "sugar" bouillon to show up these characteristics.

T. lacticolor (Gypseum group) has a nodular organ (Chlamydospores), which has a spiral form.



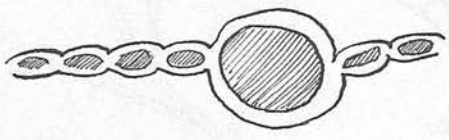
These organs give rise to new filaments.

T. niveum. This species does not show spirals, spindles, or nodular organs. It only has sporing hyphae which are simple and long.

Megaspore group. This group resembles T. acuminatum and niveum.

Faviforme colonies.

In culture the mycelium is like that found in scales and hairs, no well formed sporulating bodies - only large chlamydospores (intercalary).



T. /

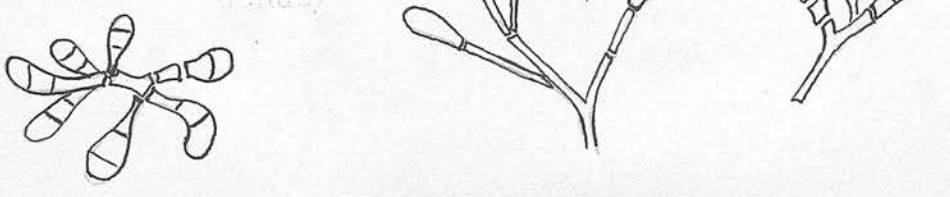
Tr. album and Tr. ochraceum present this type. They grow badly and do not show differentiating bodies. T. violaceum presents similar features.

Genus Epidermophyton.

Principal Species	E. cruris or Inguinale E. perneti. E. rubrum.
Less Known Species	E. interdigitale. E. plurizoniforme. E. lanosum. E. salmoneum.

The species of this genus have well marked characters in hanging drop preparations. There are no lateral conidia, no sporing hyphae, and no spirals, only numerous spindles with aerial branches being present. These spindles are different from those of T. gypseum and the microsporums.

The spindles of epidermophyton are lateral or terminal . The "dust" of the culture is composed of spindles.



Genus Endodermophyton.

Species: E. tropicale.
E. indicum.
E. concentricum.
E. mansoni.

These fungi in their cultural appearances are closely allied to achorions, and are difficult to grow. Reproduction takes place by sprouting and branching. No conidia-bearing hyphae are present. The fungus grows between the superficial and deep layers of the epidermis, does not invade the hair follicles, and does not cause suppuration.

II. PLEOMORPHISM.

Most dermatophytes cultivated on sugar media undergo a curious pleomorphic transformation as they grow old. The cultures develop a white downy appearance which is apparently due to degeneration of the mother culture, with the development of sterile mycelial threads. This pleomorphic phase is irreversible and a pleomorphic culture never regains its original character. Different species show variations in their capacity to exhibit pleomorphism, and a few never show this change (e.g. M. audouini). A pleomorphic culture can produce experimental lesions in animals as easily as the mother culture. A non-sugar containing medium consisting of 3 per cent. peptone and agar will prevent the development of pleomorphism indefinitely.

Apart from pleomorphism the appearance of cultures may deviate from the normal in other ways. Colonies may become powdery as a result of excessive spore formation. Pigment formation may be variable in amount, and may vary in different parts of the same colony. The presence or absence of/

of a sexual phase may considerably alter the cultural appearance and morphology of a single species. In hanging drop preparations a single spore or two spores of the same sex may be inoculated with the result that only asexual reproduction occurs in a species which, given the opportunity, is capable of sexual reproduction. The morphology of the species is modified accordingly, and only conidia are found, whereas in a perfect cycle ascospores are produced.

III. VIABILITY OF FUNGI.

Fungi are comparatively speaking hardy organisms, and display considerable powers of resistance to physical and chemical reagents. Mitchell (7) recommends allowing scales removed from lesions to dry for several days before planting, in order to minimise secondary infection. He obtained a culture of E. inguinale from scales which had been collected six months and ten months previously. Tissue which had yielded E. inguinale on culture failed to do so two, three, four and five years subsequently. Fungus infected Hair collected two years previously gave a positive culture. Mitchell obtained a culture of E. inguinale from material which had been brought to the boiling point with 15 per cent. sodium hydroxide solution. Dold (8) secured cultures of E. cruris after the material had been dried up to thirty days. Farley (9) obtained a growth of E. cruris in material which had been preserved in envelopes for 432 days. Weideman (10) obtained cultures of Tr. interdigitale for eight months from material kept in a test tube. He was unable/

unable to culture fungus from muslin which had been applied to a fungus infected area.

Weideman found that most cultures of the hyphomycetes were killed by exposure to a temperature of 51°C. for 10 minutes, but that discrepancies occurred. The resistance of fungi to cold has not so far been investigated. Chavarria, Pena and Clark (11) investigated the reaction of pathogenic fungi to X-rays and ultra-violet light. They reached the following conclusions:- (1) The roentgen-ray, up to 10 skin doses has a slightly stimulating action on pathogenic fungi of the skin; (2) visible and near ultra-violet light together are stimulating in moderate doses, but have a slightly inhibitory effect in heavy doses on non-pigmented fungi; (3) non-pigmented fungi may be sensitised by eosin, so that they are readily killed by visible light; (4) far ultra-violet light has a strong lethal action on non-pigmented fungi; (5) when a fungus develops pigment under ultra-violet light, a long exposure is necessary to produce lethal action. Sub-lethal doses result in stimulation provided the doses are short enough. Pigmentation appears to regulate the absorption of energy and is thus a defensive mechanism which favours stimulation and/

and prevents lethal action. Nadson and Phillipoo(12) found that radiation with X-rays was deleterious to the formation of sexual forms in certain fungi when given in large doses, while in small doses it exerted a stimulating action.

Kingery and Adkisson (13) tested the "in vitro" fungicidal and fungistatic properties of several volatile oils and other chemical substances commonly used in the treatment of epidermomycotic lesions. The investigations were carried out on a variety of strains of microsporum, trichophyton, achorion and yeast-like fungi. Thymol, oil of cloves and oil of cinnamon in very dilute aqueous solution (1:2000 to 1:7000) were found to be efficient, while iodine, benzoic acid and salicylic acid were found to be much less so (1:2000). Sulphur and chrysarobin were very inefficient (less than 1:50). Gould and Carter (14) found a combination of salicylic and benzoic acids to be fungistatic for Tr. interdigitale, Tr. purpureum, and Tr. gypseum in the respective concentrations of 1:30,000 and 1:22,500. Fresh cultures were found to be slightly more resistant to these substances than older/

older cultures. They showed that the fungistatic properties of these acids depended on the negative ion, and not on alterations in the pH of the medium. Schamberg and Kolmer (15) failed to show such marked fungistatic properties for salicylic and benzoic acids tested separately on different species of fungi.

In such experiments the strain of the organism, and its age probably greatly influence the results obtained so that only broad conclusions are permissible.

Chambers and Weideman (16) demonstrated an interesting biological phenomenon in connection with interdigital foot ringworm. They found that B. subtilis could be isolated from between the toes in a larger proportion of normal individuals than of individuals affected with interdigital ringworm, and also that by inoculating fungus and B. subtilis together on the same culture medium, only a growth of B. subtilis was obtained. They also found that the application of a broth culture of B. subtilis to epidermomycotic toe lesions caused marked clinical improvement in these. They suggest that B. subtilis exerts a strong anti-biotic action on fungi and that its presence on the skin may act as a preventive to fungus infection.

IV. FUNGUS DISEASES.

Only those fungus diseases which have a definite importance in dermatology as practised in temperate climates will be considered in detail, and the doubtful infections attributed to budding or yeast-like fungi will be omitted. Microsporon, trichophyton, and epidermophyton infections will be considered, and will be discussed from the clinical, therapeutic or biological aspects according to the relative importance which these are assuming at the present time.

1. Tinea Capitis.

(a) Epidemicity.

The clinical appearances of microsporon and trichophyton infections of the scalp have long been recognised, and therefore require no mention here. (Fig. 1). The varying distribution of the different species according to geographical localisation is also common knowledge. With regard to the infectivity of the disease and its occurrence in epidemic and endemic/

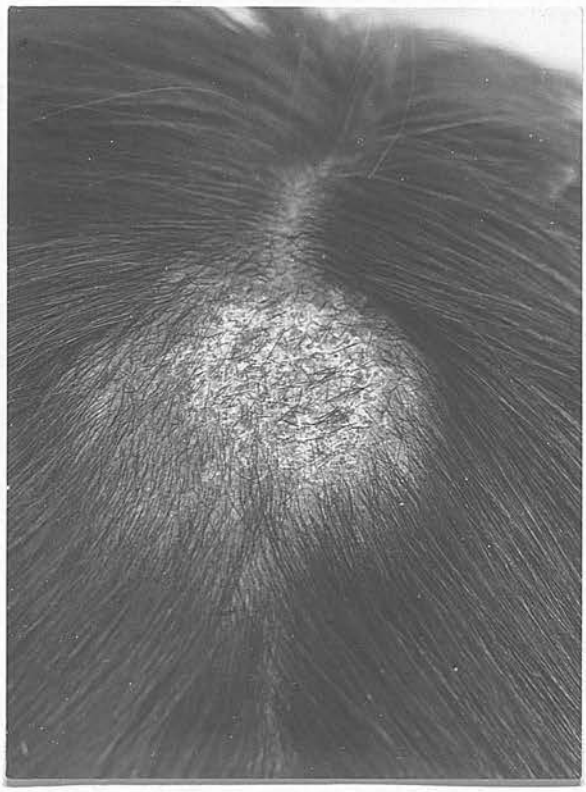


Fig. 1. Tinea capitis.

endemic form, the following charts showing the incidence in Edinburgh school children for the past eighteen years are of interest.(Charts I, II, III).

It is extremely difficult to interpret these curves and any conclusions drawn from them must be guarded. Before making any comment it must be emphasised that school inspection is carried out continuously throughout each of the three terms, and has not been concentrated on any particular period of the respective terms. During the war school medical inspection was less rigorous than during the post war period.

The curve of yearly incidence of Tinea capitis shows that the disease remains endemic, in spite of strict medical inspection and supervision of treatment. There appears to have been a wave of increased endemicity during the years 1918-1924. This may of course be more apparent than real since the figures from 1912-1917 may well be below the actual numbers as the system of isolation up to that time was an innovation. In view of the possibility of a naturally occurring periodicity in the case incidence despite isolation and supervision, it cannot be concluded that the decreasing incidence/

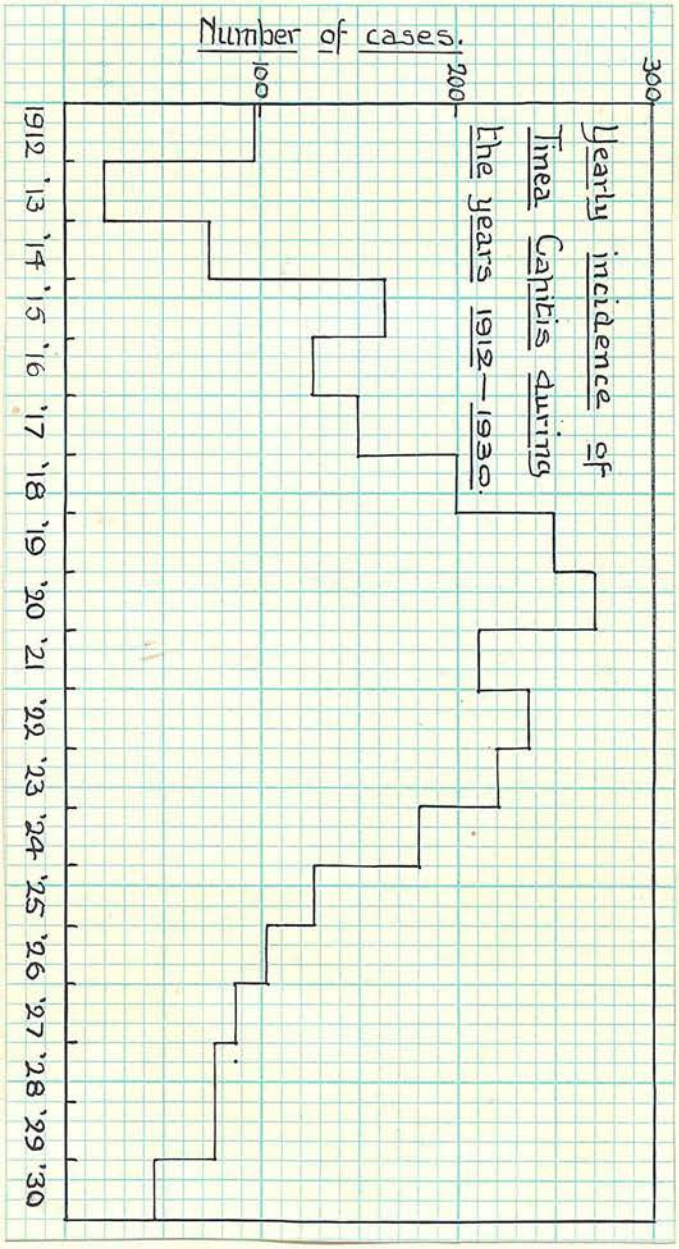

Yearly incidence of Tinea Capitis during the years 1912—1930.
Number of cases.
300
200
100
1912 '13 '14 '15 '16 '17 '18 '19 '20 '21 '22 '23 '24 '25 '26 '27 '28 '29 '30

Chart I.

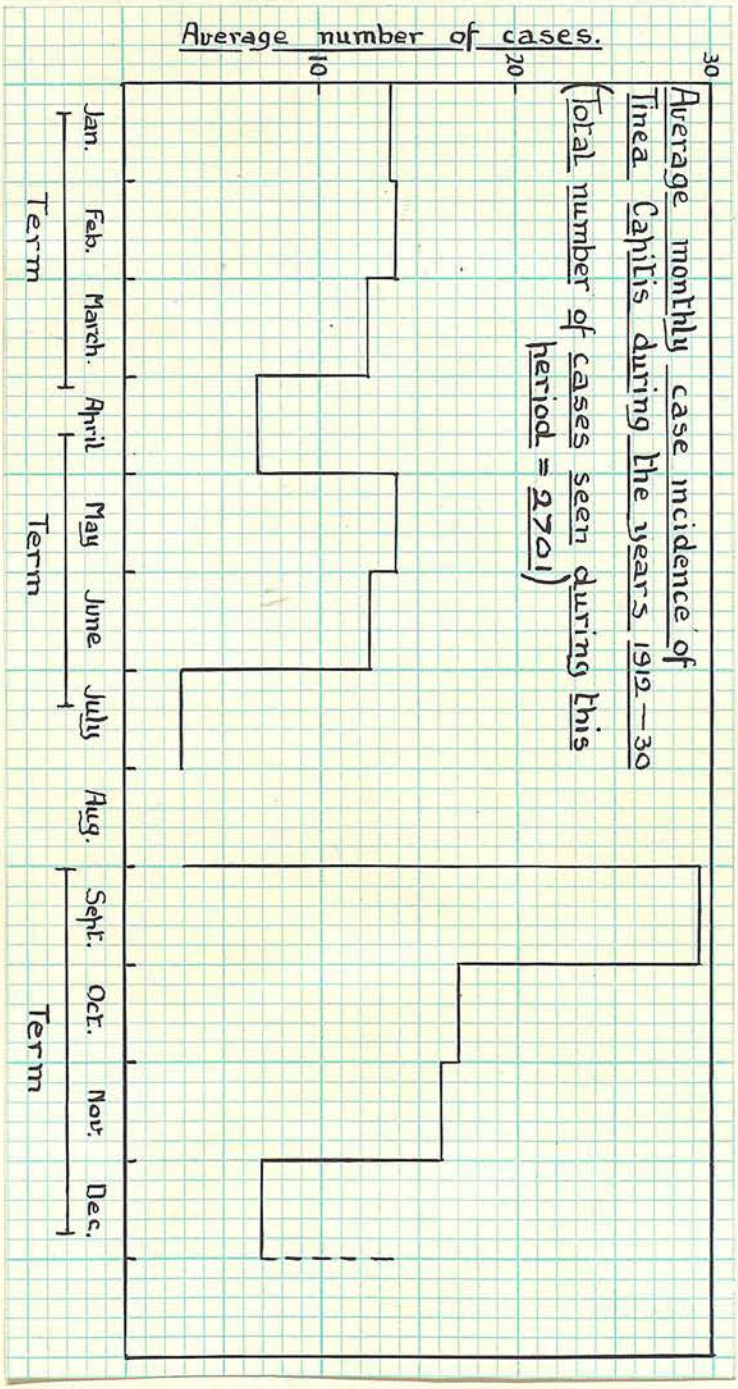

Average monthly case incidence of Tinea Capitis during the years 1912—30
(Total number of cases seen during this period = 2701)
Average number of cases.
10
20
30
Jan.
Feb.
March.
April
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Term
Term
Term

Chart II.

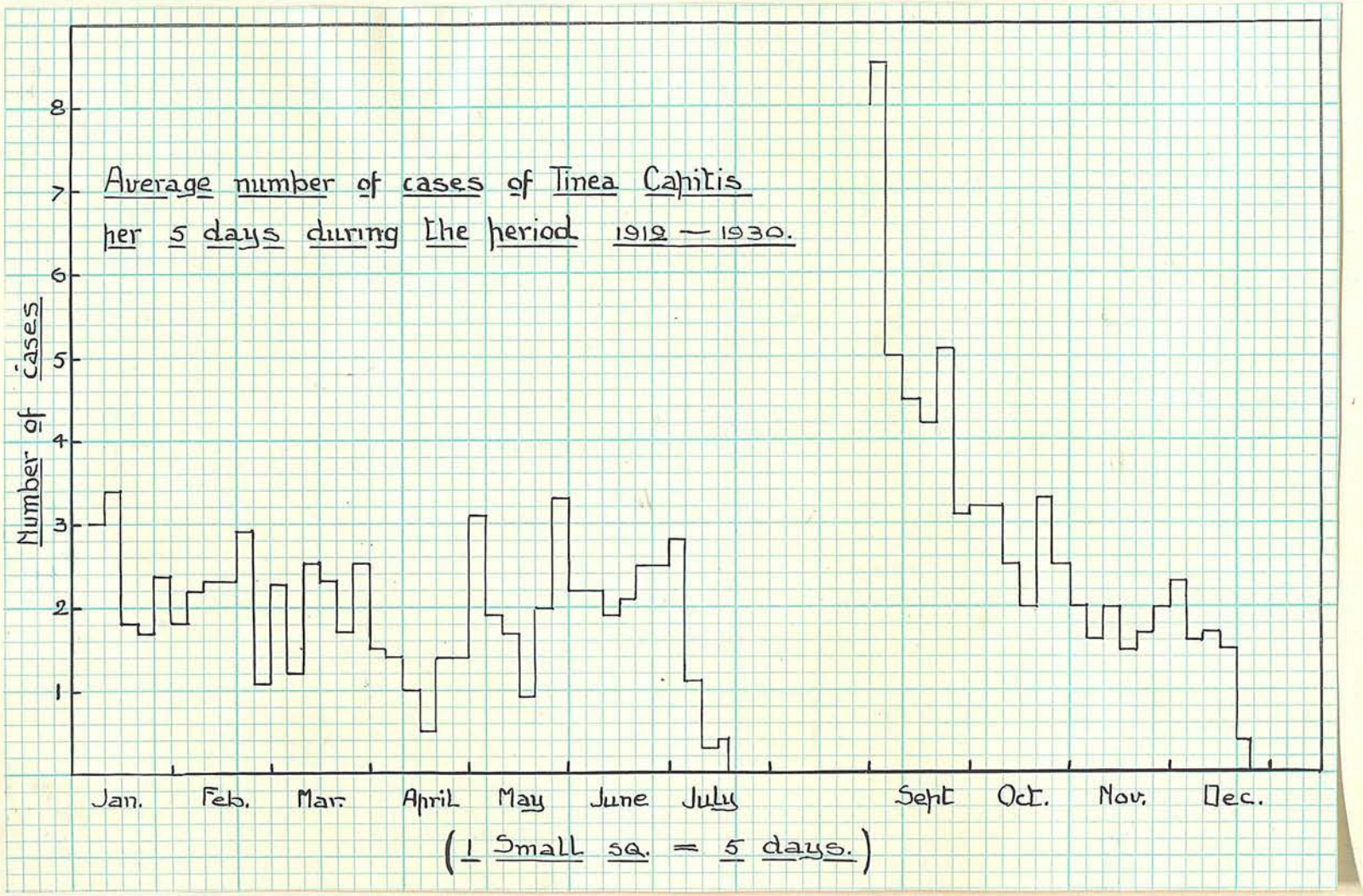

Average number of cases of Tinea Capitis per 5 days during the period 1912 — 1930.
Number of cases
8
7
6
5
4
3
2
1
Jan.
Feb.
Mar.
April
May
June
July
Sept
Oct.
Nov.
Dec.
(1 Small sq. = 5 days.)

Chart III.

incidence during the years 1925-1930 indicates the slow elimination of the disease as a result of preventive measures. As far as the available figures show, the disease is exactly comparable to scarlatina or diphtheria as regards its occurrence over a period of years.

The monthly and five day incidence charts show clearly that the isolation of cases as they occur has a beneficial influence on the case incidence, or number of contact infections, during the school terms. This is so in spite of the fact that most of the children are highly infectious for at least a few days before they are isolated, during the period when the lesions are developing to recognisable dimensions.

The highest figures of incidence are reached at the commencement of the school terms, after a period during which fresh cases probably escape detection for a longer time than is the case during the school terms when the children are subjected to routine medical inspection. The magnitude of the rise in case incidence following a vacation seems to vary directly with the length of/

of the vacation. It is recognised that in a given community of children infection may be acquired from outside sources during a vacation as a result of temporary changes of abode, or of temporary changes in the distribution of the general population. Infections acquired in such a manner cannot, however, account for more than a small increase in case incidence in any one school population, and the increase noted can only be attributed to the natural spread of the disease as a result of the absence of any controlling measures.

This fact raises the question whether infection is more readily acquired in the home surroundings than at school, and the curve of incidence can be to a great extent accounted for by the former hypothesis. That cases of Tinea capitis which are already under treatment do not act as potent sources of infection is shown by the decrease in case incidence which occurs during school terms, although the infected children under treatment are only segregated during school hours.

It may therefore be concluded that the present system of school medical inspection and treatment in Edinburgh serve to maintain Tinea capitis at a lower/

lower endemic level than would otherwise be the case if such supervision was not practised. It is possible that the system would yield better results if inspection was specially concentrated at the end of every term, and if the treatment of already recognised cases was more thoroughly supervised during the vacations.

(b) /

(b). Thallium Treatment.

With the introduction of thallium a distinct advance has been made in the treatment of microsporon and trichophyton infections of the scalp occurring in epidemic form. Soon after the discovery of thallium by Crookes in 1861, this metal was used therapeutically to diminish the night sweats of phthisis, and in the treatment of syphilis, gonnorrhoea, typhus, and enteritis. It was then noted that the drug possessed a powerful toxic action, and also that its administration was sometimes followed by a temporary loss of the scalp hair (Combemale (17), Jeanselme (18), Giovanni (19). In 1897 Sabouraud[20] realised the possibility of utilising this depilatory action in the treatment of ringworm of the scalp. Although he successfully produced epilation in several cases he encountered instances of severe toxic symptoms before he had standardised the dosage, and on this account he abandoned his researches. His reason for discarding the drug is tersely stated in the following sentence: "Si la teigne avait l'importance de la syphilis, la question/

question mériterait assurément d'être reprise... mais... il ne paraîtra prudent de risquer une néphrite pour guérir une teigne". Thereafter thallium ceased to be employed therapeutically for a number of years although Buschke (21) investigated its action on animals, and in 1905 Vignolo-Lutati (22) recorded histological observations of its action on the hair follicles. In 1918 the depilatory action in humans was again investigated by Cicero (23) in Mexico. This author was successful where his predecessors had failed, and was able to standardise a satisfactory technique for the administration of thallium salts to children, so that a single dose produced complete epilation of the hair of the scalp. Since then the drug has been used extensively in Mexico, Germany, Italy, Russia, Britain, and lately in the United States, in the treatment of ringworm of the scalp, and there are now published records of some 3,000 cases.

Thallous acetate, the acetate of the protoxide of thallium, usually referred to simply as thallium acetate, is the salt which is used to produce epilation. The dose varies from 8-9 mgm. per kilo body weight according to different investigators, and/

and the total dose calculated on this basis is administered at one time dissolved in water and given with milk. Loosening of the hair occurs as a rule from the 15-20th day after the administration of the drug, but may occur as early as the 5th or as late as the 22nd day. Mild toxic symptoms are regularly observed in a proportion of children who have received an epilating dose of thallium. These take the form of muscular pains of a pseudo-rheumatic character in the lower limbs, lethargy, and slight headache, but all of these phenomena are transitory and are completely recovered from in a few days' time. The larger doses which are required as puberty is approached are more apt to produce such toxic symptoms and for this reason the drug is not as a rule given after the age of eleven. The loosened hair can be epilated with ease, and epilation occurs to a certain extent as a result of washing the scalp. To obtain a completely bald scalp, however, systematic removal of the loosened hairs by hand, forceps, or plaster is necessary. Once epilated, the scalp remains bald for about two weeks and then a regrowth of hair becomes apparent, and a good growth usually occurs in from 3-4 months from the date of administration of the drug. Only the hair of the scalp/

scalp is affected with the dose stated; the eyebrows, eyelashes and nails are unaffected.

Since November 1928, 139 children suffering from ringworm of the scalp have received an epilating dose of thallium acetate at the Skin Department, Royal Infirmary, Edinburgh. The treatment has been successfully completed in 122 of these cases, the remaining 17 being still under supervision. During the same period only 29 children suffering from ringworm of the scalp have received an epilating dose of X-rays.

In the cases under consideration the treatment consisted in the oral administration of thallium acetate in a single dose of 8.5 mgm. per kilo body weight, and the daily application to the scalp of a 10 per cent. sulphur ointment. When a good regrowth of hair had taken place local treatment was suspended for three weeks, at the end of which time the scalp was examined for any evidence of disease.

A series of fifty consecutive cases which had received thallium has been carefully observed and subjected to special investigations, with a view to gaining some idea of the mode of action of thallium. For convenience the cases are recorded in tabular form.

Table/

TABLE. 1.

Age-period. Years.	Case No.	Sex.	Weight in kilos.	Day on which hair loose.	Day on which epilation was complete.	Duration of toxic symptoms.	Day on which re-growth commenced.	Result of treatment.
1–2	44	M.	11·0	17th	20th	None	..	D. 100*
3–4	19	M.	14·24	17th	22nd	8th–10th day	?	D. 114
4–5	16	F.	15·32	..	20th	None	?	Not cured.
5–6	1	F.	17·42	18th	20th	7th–11th day	?	D. 123
	43	M.	19·6	15th	20th	None	30th	D. 106
	5	M.	17·36	..	21st	7th–21st day	..	D. 124
	12	M.	18·44	16th	20th	7th–10th day	29th	D. 106
	20	M.	18·24	17th	21st	None	34th	D. 98
	22	M.	18·16	16th	20th	,,	32nd	D. 114
	23	M.	18·28	..	20th	7th–13th day	34th	D. 148
	49	F.	14·4	..	..	None	..	D. 96
	25	M.	18·04	13th	20th	,,	33rd	D. 63
	28	M.	17·4	15th	20th	,,	28th	D. 130
	41	M.	16·82	17th	20th	,,	..	D. 112
6–7	7	F.	16·2	17th	20th	6th–13th day	27th	D. 112
	8	F.	20·72	..	20th	13th–16th day	29th	D. 104
	13	M.	17·50	16th	20th	None	28th	D. 106
	31	F.	18·16	..	..	13th–16th day	..	D. 95
	33	M.	15·46	..	..	None	..	D. 100
	36	M.	19·0	..	..	13th–18th day	..	Not cured.
	37	F.	19·1	..	..	None	..	D. 119
	38	M.	15·9	..	..	,,	..	D. 112
	39	M.	17·0	17th	17th	4th–8th day	20th	D. 122
	40	M.	18·26	17th	20th	None	..	D. 112
7–8	2	F.	18·02	18th	20th	,,	33rd	D. 130
	45	M.	22·56	12th	18th	,,	..	D. 90
	6	M.	18·74	18th	20th	,,	30th	D. 114
	50	F.	26·8	15th	20th	,,	..	D. 96
	9	F.	21·4	16th	18th	6th–13th day	..	D. 104
	10	M.	19·12	2nd	20th	8th–13th day	29th	D. 146
	11	M.	19·28	17th	20th	6th–8th day	28th	D. 122
	15	F.	22·42	..	19th	None	30th	D. 114
	18	F.	20·88	..	20th	,,	30th	D. 94
	24?	M.	15·4	..	21st	,,	30th	D. 63
	29	M.	21·04	15th	20th	13th–15th day	28th	D. 65
	47	M.	21·2	16th	20th	None	..	D. 98
	32	M.	19·04	..	..	8th–15th day	..	D. 95
	35	M.	16·4	..	..	11th–14th day	..	D. 118
	42	M.	22·56	..	20th	None	30th	D. 112
8–9	14	M.	23·36	16th	18th	,,	27th	D. 105
	46	F.	22·8	19th	21st	,,	..	D. 110
	17	F.	22·24	..	20th	10th–14th day	36th	D. 114
	48	M.	16·25	..	18th	..	..	D. 98
	26	F.	22·0	15th	20th	15th–18th day	28th	D. 123
	30	F.	22·1	..	..	13th–15th day	..	D. 95
	34	M.	24·5	..	..	3rd–18th day	..	D. 95
9–10	3	F.	25·14	..	..	10th–28th day	30th	D. 106
	21	M.	23·94	16th	18th	None	?	D. 114
	27	M.	25·0	15th	21st	,,	28th	D. 123
10–11	4	M.	23·88	..	..	6th–25th day	28th	D. 85

* D. 100 = Discharged cured 100 days after thallium administration.

Serum calcium estimations were performed in Case 8, and the following results were obtained:

Immediately prior to thallium administration	10.3 mgm. Ca/c.c.	serum.
10th day after thallium do.	10.4 "	"
21st day after thallium do.	10.0 "	"

Case 10 received 2 gr. of thyroid extract daily from the day on which thallium was administered until epilation was complete.

In Case 11, 3 gm. calcium chloride were administered daily during the first 20 days after the dose of thallium. Electrocardiographic records were obtained at intervals in Case 33, and it was found that thallium did not influence the normal electrocardiogram.

The effect of thallium on the basal metabolic rate was investigated in cases 6, 13, 33, 36, 38, 39, 40, 45, i.e. in 8 male children in the age group 6-8 years. These children were divided into two groups of four. In the first group the B.M.R. was determined and an epilating dose of thallium was then administered immediately. B.M.R. estimations were again performed on the 10th and 20th days after thallium administration. In the second group the B.M.R. was determined and then thyroid extract was administered in 1 grain doses daily/

daily. On the 14th day the B.M.R. was again determined, thallium was administered, and the thyroid extract was continued. B.M.R. estimations were repeated on the 10th and 20th days after thallium administration. For two mornings prior to the actual estimation of the B.M.R. the mask was applied to the face for a short period in order to accustom the child to the procedure and to eliminate as far as possible any error in the result due to fright or nervousness. The results are shown in Charts IV and V.

It will be seen that in both groups there is a drop in the B.M.R. following thallium administration. In the first group (thallium alone) the average drop during the 20 days following thallium administration is 23 per cent; in the second group (thallium + thyroid) the average drop during the same period is 11.25 per cent.

Case 19 is of special interest as it throws some light on the mode of action of thallium which will be discussed later. Case 19, was a boy, aged 3½ years, suffering from a small spored ringworm of the scalp, had had a pigmented and hairy naevus on the left cheek since birth. This naevus involved the left temple and lateral half of left eyebrow, the/

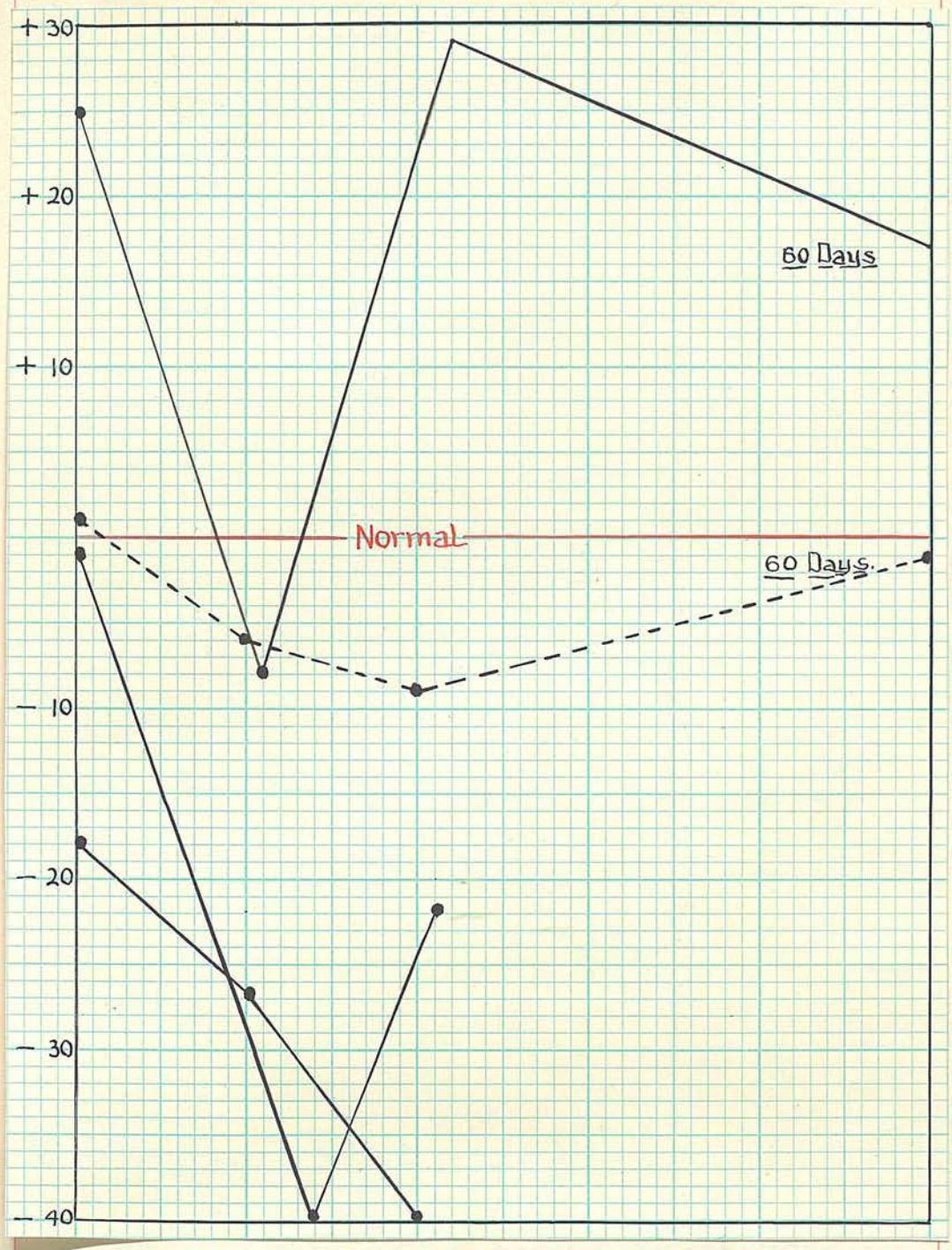


Chart IV. The effect of an epilating dose of thallium on the basal metabolic rate in children.

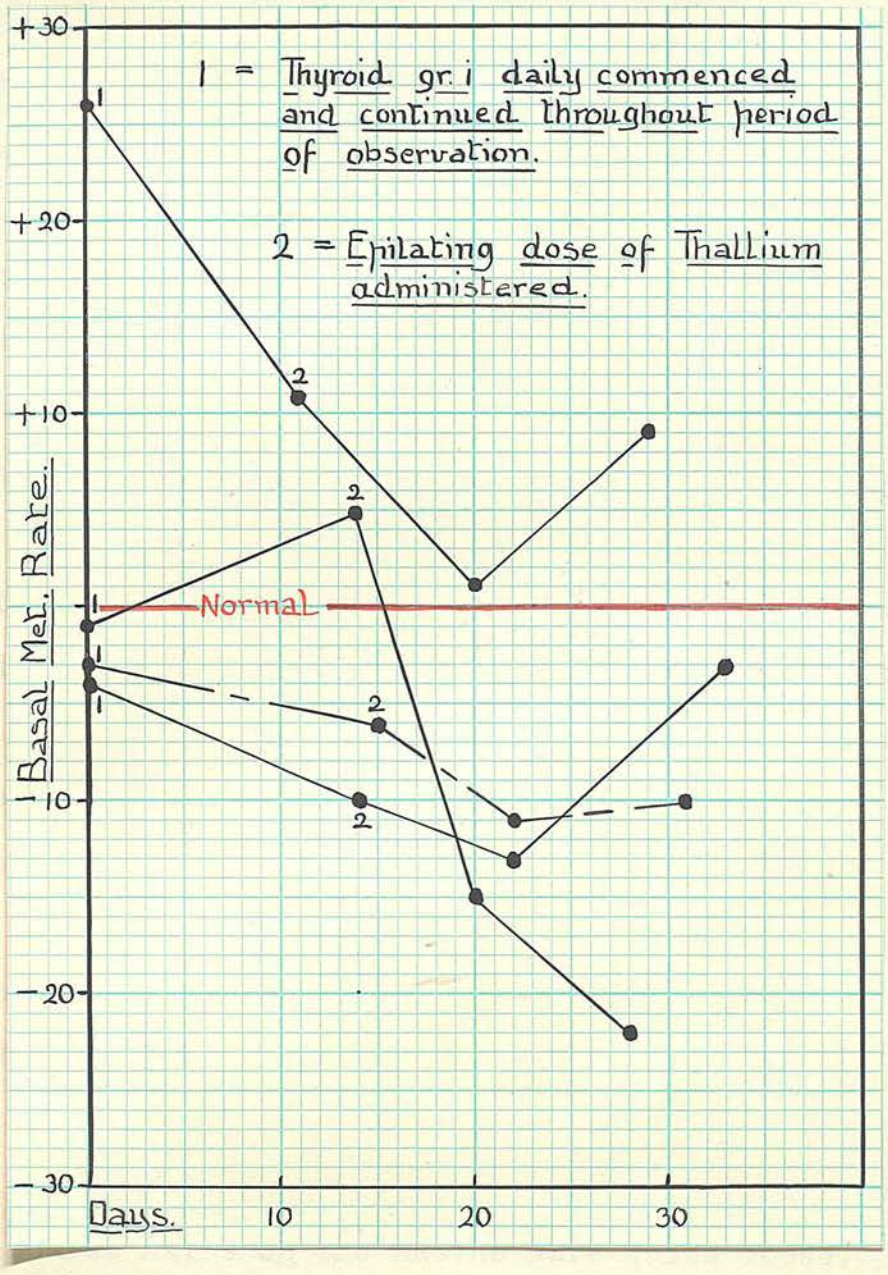


Chart V. The effect of an epilating dose of thallium on the basal metabolic rate in children receiving thyroid extract.

the left malar region, left eyelid, and left side of the tip of the nose (Fig. 2). It was covered with a fine, short, white, downy growth, and numerous coarse, strong, rapidly growing hairs, about 1 to 2 inches long were scattered over the entire surface. All the hairs were firmly fixed in their follicles, and considerable traction was required to pull them out. This procedure was decidedly painful, and was objected to strongly by the patient. That portion of the naevus situated on the nose was slightly verrucous on the surface. Thallium acetate was administered in a dose of 8.5 mgm. per kilo body weight, and, calculated on this dosage, 0.113 grams were administered in a single dose by the mouth. Seventeen days after the administration of thallium the scalp hair was loose in places, and some of the coarser hairs on the naevus could be pulled out easily and painlessly. On the 14th day, the coarse hairs were loose over the entire surface of the naevus. On the 18th day, all the coarse hairs on the naevus were quite loose. The patient pulled them out of his own accord, and on that day all the remaining coarse hairs were epilated. Complete epilation was obtained on that portion of the naevus involving the left eyebrow, and over an area two inches in diameter situated in front of the left ear. Over the remainder of the naevus, a fine, white/

Fig. 2. Naevus pilosus et pigmentosus before administration of an epilating dose of thallium.

white, downy growth persisted. Since the administration of thallium, these downy hairs had always been firmly fixed in the follicles (Fig. 3) On the 22nd day, depilation of the scalp was complete. Neither depilation nor loosening of the hair occurred on the right eyebrow or the inner half of the left eyebrow. No toxic symptoms were observed to follow the administration of thallium, and the weight remained constant. Apart from the depilation of the coarse hairs, no other phenomena were observed to occur on the area involved by the naevus.

Hair growth proceeded normally for a surprisingly long period during the interval which elapsed between the administration of thallium and the occurrence of depilation. To illustrate this point, portions of the scalp were shaved in several cases at the time of administration of thallium, and later re-shaved at varying intervals. The results are shown photographically in Fig. 4. In almost all cases both long and short hairs, obtained at the time of epilation, were examined microscopically. The hair-bulb was usually normal in shape, but in a certain number of cases showed deformities, such as thinning, tapering, and twisting on/

Fig. 3. Naevus pilosus et pigmentosus 19 days after an epilating dose of thallium.

Fig. 4 (a). Case 72.

This child received an epilating dose of thallium 6 days before the front of the scalp was shaved.

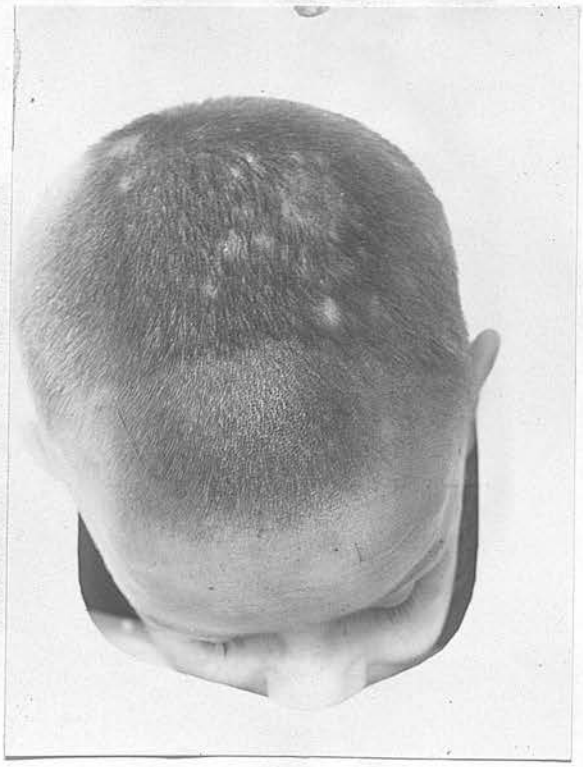


Fig. 4 (b). Case 72.

Photograph taken on the 11th day after administration of an epilating dose of thallium, demonstrating the persistence of growth power in the hair matrix between the 6th and 11th days after thallium.

Fig. 4 (c). Case 72.

Complete epilation on the 18th day after thallium administration.

on itself. No marked disturbance of pigment distribution, such as occurs in alopecia areata, was noted after thallium administration. In practically every instance the hair-bulb and shaft were normally pigmented. Occasionally the hair-shaft showed a greater amount of pigment in its upper portion than in the bulb, but in no case was the transition sudden, nor was there any massing of pigment, clubbing, or fragmentation at the free end of the short hairs, such as is observed constantly in the short exclamation-mark hairs of alopecia areata. The microscopic appearances of thallium hairs are shown in Fig. 5 along with those of exclamation-mark hairs (Fig. 6) for purposes of comparison.

The shafts of the long hairs occasionally presented a slight moniliform appearance, but this always occurred in the neighbourhood of the extremity, and must have been produced at some time prior to the administration of thallium. In all cases the free end of the shaft was cut sharply across, and it is probable that the variation in length of the hairs was due to variation in the growth power of the hair-bulbs at the time of the last/

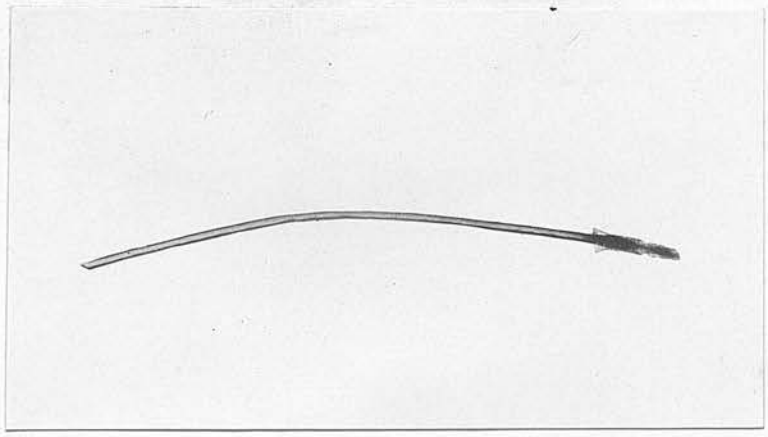


Fig. 5 (a). Thallium hair, showing well formed pigmented root and square cut tip.

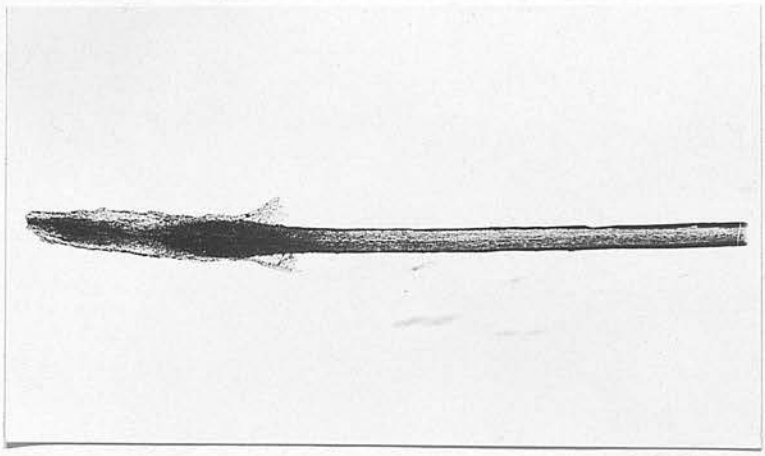


Fig. 5 (b). Thallium hair, showing well formed pigmented root and shaft.

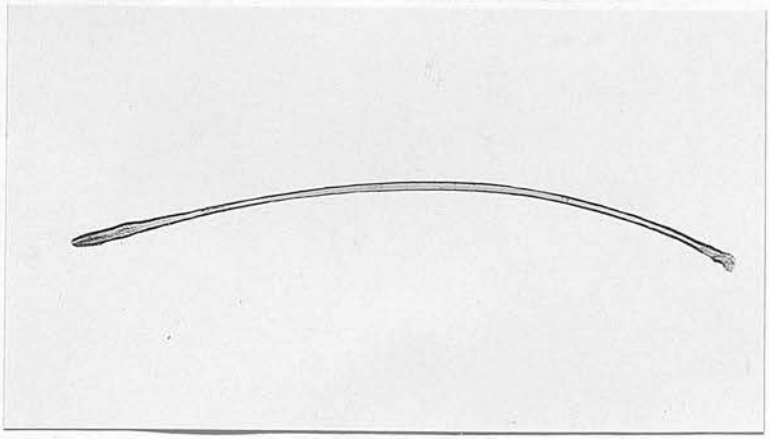


Fig. 6 (a). Exclamation mark hair, showing bulbous pigmented tip, depigmented shaft, and atrophic depigmented root.

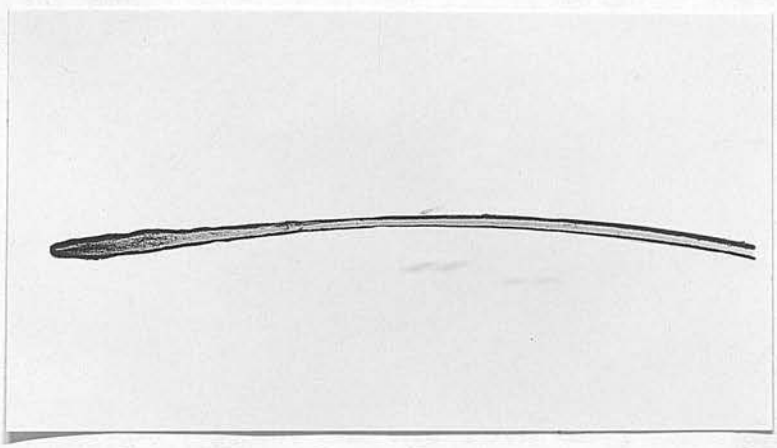


Fig. 6 (b). Exclamation mark hair, more highly magnified, showing bulbous pigmented tip and depigmented shaft.

last clipping.

In every case the hair grew in satisfactorily after the temporary alopecia due to the thallium. At first the growth was sparse and the hairs of a somewhat coarse texture. Later the growth became thicker, and the hairs resumed their normal texture. In many cases the regrowth of hair, which prior to the administration of thallium had been straight, took the form of profuse curls (Fig. 7), a phenomenon commonly observed to follow epilation produced by X-rays.

From the foregoing results it will be seen that successful epilation of the scalp occurred in every case. This was unaccompanied by a fall of hair on other parts of the body or by lesions of the nails. Epilation was complete in from 17 to 22 days after the administration of the drug. Uruena (24) found that in the great majority of his cases epilation took place on the 15th day, but observed cases in which it occurred as early as the 5th day and as late as the 22nd day. He encountered only 9 thallio-resistant individuals in his series of 612 cases. The same author states that girls are more susceptible/

Fig. 7. Regrowth of curls following thallium administration. Prior to thallium the hair was straight.

susceptible to thallium than boys, and that in the former epilation occurs earlier, but no such sex difference was noted in this series.

The percentage cures in any series is difficult to estimate, as many cases are lost sight of. The figures recorded by different authors vary from 50-91% cured, while those in which treatment was definitely unsuccessful range from about 8-27%. The thoroughness of local treatment during the period in which the scalp is completely bald is of first importance in the ultimate cure of the disease. In the present series, the local treatment was carried out daily by skilled nurses, and a cure was obtained in 96% of cases. I have obtained similar results in a further series of 72 cases. In the whole series of 122 completed treatments, only two cases failed to be cured after one administration of thallium, the reason being incomplete epilation and careless local treatment due to intercurrent illness. The cause of the incomplete epilation was presumably incomplete absorption of the quantity of thallium administered. These two cases were given a second dose of thallium after a period of 6-8 months had elapsed from the time of the previous administration.

No/

No undue toxic phenomena followed the second dose of thallium.

Observations on the effect of thallium on the hair-shaft and bulb, which are similar to those here recorded, have been made by Uruena, Fiocco (25), Vignolo-Lutati (22), Balbi (26), and Jeanselme (18). These authors noted deformity and atrophy of the hair-bulb, but did not observe any constant alteration in pigment distribution. On the other hand, Pardo-Castello (27) states that short thallium hairs resemble exclamation-mark hairs, and his suggestion of the close identity of thallium alopecia and alopecia areata is partly based on this statement. The illustration of a short thallium hair which accompanies his article shows a well-pigmented shaft with a square-cut free end. The exclamation-mark hair in alopecia areata shows quite the reverse type of pigment distribution, together with a distinct thinning of the lower part of the hair-shaft and clubbing and fragmentation of the free end.

It is to be noted that the administration of thyroid extract and calcium chloride respectively to two cases did not in any way interfere with the depilating action of thallium, and the calcium content/

content of the serum was found to remain within normal limits following thallium administration.

The estimation of the basal metabolic rate in children is attended with greater difficulties than are met with in adults. Accurate charts of surface area are not available for children, and on account of their natural restlessness and the nervousness induced by the necessary manipulations of the technique, it is difficult to maintain basal conditions. Any figures for the basal metabolic rate must therefore be interpreted with caution. In the present series thyroid was administered to one group of children to see if it would counteract the tendency to the lowering of the B.M.R. following thallium administration which preliminary experiments had indicated. A comparison of the figures obtained in the two groups suggests that a fall in the B.M.R. does occur after thallium. The coincident administration of thyroid tends to diminish this fall, even although thyroid administration during the preliminary period of 14 days prior to thallium administration only produced a slight rise in the B.M.R. in one case, and in three was accompanied by a fall. The lowering of the B.M.R. after thallium/

thallium may be due to two causes: (1) the action of thallium on the thyroid gland, (2) the general toxic action of thallium causing lethargy and lessened movement. The subsequent rise in the B.M.R. is rather against the view that the fall is due to the patient becoming accustomed to the method of estimation and that a more restful or basal condition is attained as a result of previous experience of the procedure.

Toxic symptoms occurred between the 3rd and 28th day in 44% of the cases under consideration. These consisted of muscular pains in the legs of a pseudo-rheumatic character, lethargy and slight headache, and lasted from 2 to 19 days. In no case were the symptoms severe, and few of the patients were even confined to bed. No age-period was specially liable to show toxic symptoms, although it is generally stated that the age of the patient appears to influence their occurrence. Toxic phenomena were observed by Lourier and Zwitkis (28) in 19% of the children in the age-period 1-2 years; in 9% between 3 and 7 years; and in from 12-25% between 8 and 18 years.

There are about 25 recorded cases of death due/

due to very gross overdosage of thallium acetate, either accidental or criminal. Lynch and Scovell (29) report three cases of accidental thallium poisoning in which ten times the epilating dose was given. The children died in from 30 hours to 3 days with symptoms of collapse and central nervous system paralysis. The post mortem findings were marked fatty degeneration in the heart and liver, and necrosis in the kidney, and resembled those resulting from phosphorus poisoning. Thallium was recovered from the stomach, liver, intestines, kidneys and urine in all three cases. The amount of thallium recovered in these organs in the case which survived for 3 days was much less in proportion to the total dose administered than in those which died earlier, suggesting that thallium is eliminated in appreciable amounts soon after its administration. They quote Wilcox as having seen cases of poisoning followed by recovery in children who had received a full epilating dose weekly for 2-3 weeks. The symptoms observed included nausea, collapse and convulsions. Davies (30), Fuld (31) and Ritter and Karrenberg (32) each report a case in which/

which the administration of an epilating dose of thallium was followed in 2-14 days by vague symptoms of gastric and central nervous system disturbance and temperature, but which quickly recovered. From the nature of the symptoms and the interval elapsing before their onset, and also from the fact that in one case epilation was poor, it is open to question whether the conditions described were really due to the thallium. A similar instance occurred in the present series (Case 29), where pains in the legs, nervous irritability, lethargy and abdominal pain, developed gradually from the 3rd to the 14th day after the administration of thallium. On the 14th day the patient vomited a female round worm, and the symptoms settled down immediately.

With a few exceptions all who have employed thallium, including those who support the theory of an endocrine-vegetative nervous system involvement, are agreed that the mild toxic symptoms which may follow its use are of no importance, and do not contra-indicate its administration to otherwise healthy children. There is no definite evidence to show that thallium has a dangerous toxic action, any more than is the case with arsenic, mercury,

some/

some of the alkaloids, or biological substances.

Investigation of the Subsequent Nutrition of Children who have received an Epilating Dose of Thallium.

In view of the fact that toxic symptoms do occur, that thallium is a substance capable of producing, in larger doses, profound toxaemia and grave pathological changes, and that its fate in the body and mode of action as a depilatory substance are unknown, it is possible that it may cause deleterious influences on growth and development which only become evident at some later period. This aspect of the problem is obviously a most important one if the therapeutic use of the drug is to be continued. I have therefore followed up a series of 76 male and female school children who received thallium acetate treatment for ringworm of the scalp at periods varying from four months to two years previously. Each of these children was examined physically, and all appeared to be healthy; indeed, many of the parents volunteered the statement that in their opinion the child's general health had improved after the administration of the thallium. In addition, each child/

child was measured and weighed. The group consisted of 51 males and 25 females.

First of all the children were divided into age groups according to their age on the last birthday, and the average weights of the corresponding age groups before and after thallium administration, were comparied. The average weights on these two dates do not represent the weights of the same children for any one age group, since a child aged 5 at the time of administration of thallium may have been examined eight months or two years later, in which case this child's weight would go to make up the average either of age period 5 again, or of age period 7. In spite of this, the data provide a comparative index of the nutrition of the children before and after thallium administration, since the weights before thallium treatment indicate the type of population dealt with and those after thallium deal with the same population, so that any redistribution into different age groups is immaterial. The average weights on these two dates were compared with the average normal weight of Edinburgh school children for the same age periods. The total numbers in each age group were as follows:

Table /

Table 2 .

Age last birthday	Males		Females	
	Before thallium.	4 months to 2 yrs after thallium.	Before thallium.	1 to 2 yrs. after thallium.
1	1	-	-	-
2	-	-	-	-
3	1	1	1	-
4	5	1	3	-
5	10	3	1	2
6	10	9	6	3
7	11	10	7	4
8	8	10	3	6
9	4	9	2	5
10	1	5	1	3
11		3	1	1
12		-		1
	51	51	25	25

Table 3 shows the average weights in kilograms according to age period of 51 male children immediately/

immediately before the administration of thallium, and of the same children four months to two years after they had received a single epilating dose of thallium. The average height in inches and the average normal height is also shown.

Table 3 .

Males, Four Months to Two Years after Thallium.

Age last birth-day.	Average weight before thallium.	Average weight 4 months to 2 yrs. after thallium.	Average normal weight.	Average height 4 months to 2 yrs. after thallium.	Average normal height.
1	11	-	9.5	-	-
3	13.9	16.6	14.1	38.1	35
4	15.74	15.9	15.9	35.5	38
5	18.32	17.53	18.2	41.4	41
6	19.32	20.6	20.2	44.3	44
7	22.22	22.8	22.6	46.4	46
8	23.59	25.4	24.9	49.3	47
9	24.2	25.9	27.4	49.57	49.7
10	25.4	26.6	30.7	49.6	51.8
11	-	28.3	32.7	51.3	53.5

Table /

Table 4 gives similar data for 25 female children 1-2 years after thallium.

Table 4.

Females, One to Two Years after Thallium.

Age last birth-day.	Average weight before thallium.	Average weight 1 to 2 years after thallium.	Average normal weight.	Height 1 to 2 years after thallium.	Average normal height.
3	14.4	-	12.6	-	-
4	15.8	-	15.4	-	-
5	17.9	17.9	17.8	40	40.5
6	19.1	20.0	18.9	45.5	42.8
7	20.88	23.0	21.3	46	44.5
8	23.4	23.9	23.7	47.4	46.8
9	25.5	26.2	25.2	48.4	48.7
10	26.06	28.5	28.1	52.9	51
11	28.24	31.8	30.9	51	53.1
12	-	32.7	34.7	55.5	55.6

In order to control the results of a comparison of the average weights before and after thallium, and of the average normal weight classified according to age periods, the average weights after thallium according to height were compared with the average normal weights for the same heights. Table 5 shows the average weight in kilograms according to/

to height in inches four months to two years after thallium in 51 male children, and one to two years after thallium in 25 female children.

Table 5.

Males and Females.

Height in inches.	Males		Females	
	Average weight 4 months to 2 yrs. after thallium.	Average normal weight.	Weight 1 to 2 yrs. after thallium.	Average normal weight.
35	15.9	14.1	-	-
38	16.6	15.9	-	-
40	19.1	17.4	16.3	16.8
41	16.8	18.2	21.3	20.58
43	19.8	19.4	22.2	21.8
44	20.3	20.2	22.6	22.4
45	21.2	21.3	24.8	23.9
46	21.9	22.6	27.2	25.59
47	23.8	24.9	30.7	28.1
48	24.6	25.8	30.4	29.5
49	24.4	26.7	29.0	30.9
50	26.9	28.1	32.7	34.5
51	28.1	29.5	-	-
53	28.6	32.7	-	-
55	34	35	-	-

An analysis of Tables 3 and 4 shows, first, that immediately before the administration of thallium the average weight according to age of the 51 male and 25 female children dealt with in the investigation, compares favourably with the average normal weight for the corresponding age periods. The weight of the males up to the age of 7, and of the females up to the age of 9, is practically identical with the average normal weight, but in the later age periods it is anything from 1.4 to 5 kilograms below normal. The difference is greatest in the males. It is to be noted, however, that in both cases there were fewer children in the age periods 9 and 10 years than in the age periods 4 years to 8 years. In Tables 3 and 4 the average weight of the same children four months to two years after thallium is seen to be on the whole better than that for the corresponding age periods immediately prior to its administration. Table 2 shows that this improvement cannot be due entirely to a redistribution of the children into different age periods, at least for the period six years to eight years, since/

since almost the same number of children appear in these groups before and after the administration of thallium. In Tables 3 and 4 there is a fall in weight below the average normal weight for the age periods 9, 10, and 11 years, and this corresponds to that found in the average weight, before thallium was given, for the periods 8, 9, and 10 years. The average weight of the female children one to two years after thallium administration is quite appreciably higher than the corresponding average normal weight.

Table 5 shows that in both male and female cases the height four months to two years, and one to two years after thallium administration bears a normal relationship to the weight; and Tables 3 and 4 show that the average height at a similar period after thallium administration is normal for the various age periods. In males the height for the age periods 10 years and 11 years is below the average normal, but this corresponds to the subnormal average weight for the same periods. As the period elapsing between the first and second weighings varied in most of the children, no attempt has been made to ascertain the average/

average increase in weight during this interval of children of corresponding age periods or heights.

Examined from several aspects the weights and heights of both male and female children four months to two years after thallium administration do not appear to show any gross deviation from the normal. In fact, below 10 years of age the figures are, if anything, slightly better than normal. Using the weights and heights observed in this investigation as standards on which to interpret physical development, and taking into account the results of a general medical examination, there is no evidence to show that the continued normal growth and nutrition of children are adversely influenced or interfered with as a result of the previous administration of thallium acetate in sufficient quantity to produce epilation of the scalp.

The/

The Advantages and Disadvantages of Thallium.

Thallium is easily and rapidly administered On account of this a large number of children can be dealt with in a very short time, whereas an epilating dose of X-rays for the entire scalp takes at least an hour to administer, even with appropriate apparatus and skilled technique. This fact renders thallium especially suitable when a sudden epidemic has to be dealt with. For instance, a comparison of the average period elapsing between the administration of thallium and the treatment by X-rays respectively and the discharge of the case, shows that in 122 thallium cases this period was 113.5 days; in 29 X-ray cases of one series it was 152 days, and in 50 cases of another group it was 135 days. Further, in 1923, the average period of isolation of ringworm cases treated with X-rays was seven months, this delay being due to the occurrence within a short period of time of a large number of cases which could only be dealt with gradually on account of the time occupied by the administration of the X-ray dosage. Epilation with thallium is as certain as that obtained by X-rays; there is no risk of permanent baldness, and it/

it can be produced in children who are too young to receive X-ray treatment.

On the other hand, the alopecia resulting from thallium is of much shorter duration than that brought about by X-rays, so that with thallium very careful local anti-parasitic treatment is required. Another disadvantage of thallium is that it regularly produces mild constitutional symptoms in a proportion of cases (44 per cent. in 50 of the present series). These are never serious with ordinary dosage (up to 0.25 to 0.28 gram) in children under 11, and are no more severe than those which may accompany scarlatinal or diphtheria immunization. They are quickly recovered from, and no immediate harm seems to result.

Discussion on the Probable Mode of Action of Thallium.

On account of the interval which elapses between the administration of thallium and the onset of depilation, and also in view of the apparent selectivity of the drug for certain regions, it has been claimed by several authors that the depilation is not due to the direct action of the drug on the hair/

hair-follicles, but results from the effects of an intermediate action on some other organ. It has been suggested (27, 31, 34, 35,36) that the alopecia is produced by a temporary arrest of the trophic functions of the endocrine system. The thyroid (27), parathyroids and possibly the supra-renals (33, 34, 35) have been supposed to be particularly affected, the result being the establishment of a partial syndrome of glandular insufficiency. Alterations in the activity of the vegetative nervous system are also supposed to occur in association with this condition of endocrine dysfunction, on account of the close inter-relation-ship which exists between the two systems. The evidence for these statements has been derived partly from pathological and partly from biological observations, but in neither case does it fully support the conclusions arrived at.

Thallium intoxication causes pathological changes in many organs. Uruena (24) reports inflammation of the gastro-intestinal tract with degeneration of the mucous membrane, congestion and haemorrhage in the liver, and an acute tubulo-nephritis. Buschke and Peiser (33, 34, 35) and Bernardt/

Bernardt (37) noted atrophy of the thyroid, parathyroid, and suprarenal glands and testes, together with absorption and rachitic-like changes in the bones, a high serum calcium and cataract. Leigheb(38) observed pulmonary inflammation, catarrhal and haemorrhagic enteritis, degenerative changes in the cells of the liver, pancreas, thyroid and kidneys, and congestion of the brain and meninges. Pardo-Castello (27) found congestion of the intestine and liver, atrophy of the spleen, and parenchymatous degeneration and atrophy of the suprarenal and thyroid glands. Lynch and Scovell (29) found marked fatty degeneration of the heart and liver, and necrosis of the kidneys.

These findings indicate that in toxic concentrations thallium acts as a general protoplasmic poison, causing grave damage to all the important organs. In the presence of such widespread lesions it seems unjustifiable to attribute the depilatory action of thallium to a specific effect on any individual organ which may show pathological changes following toxic doses of the drug. That such an intermediary action should be responsible for depilation is all the more unlikely, when it is considered/

considered that the temporary fall of hair produced by therapeutic doses is unaccompanied by any other symptoms in a large proportion of cases.

Buschke and Peiser(39) found that in tadpoles growth was inhibited by the administration of thallium, but that it took place normally if thyroid and thymus extract were administered in addition. This observation, however, does not furnish conclusive evidence that thallium on the one hand and thyroid and thymus extract on the other have a directly antagonistic action on the organism, and Aramaky (40) found that in rats thyroid feeding did not in any way influence the loss of hair produced by thallium administration.

The fact that in human subjects toxic symptoms due to thallium occur much more frequently and are more severe after puberty than before it, has also been quoted to support the theory of endocrine involvement. The changes in the activity of the thymus and gonads which occur at puberty may, however, be coincident with the increased frequency of toxic symptoms and yet bear no direct relationship to it. It is very probable that/

that thallium, like other heavy metals, is concentrated in certain tissues. Should this be the case, the ratio of amount of thallium to body weight may no longer hold as a therapeutic index of dosage as the weight increases with age, since beyond a certain body weight the total amount of thallium given may be too large to be distributed throughout these tissues in non-toxic concentration.

Clinical observations of the therapeutic action of thallium in children do not suggest the occurrence of any grave derangement of the endocrine glands. Apart from the occasional manifestation of mild toxic symptoms of a non-specific nature, the patient appears to be perfectly normal, and health and growth are not interfered with in any way (24,25,38, 41). The fall of scalp hair, which is the sole apparent effect of therapeutic doses of thallium, takes place suddenly, involves the entire scalp, and is soon followed by complete regeneration. This is a very regular sequence of events, and constitutes a clinical picture which is not comparable with any known syndrome of endocrine dysfunction. For example, the alopecia observed in myxoedema has a/

a slow onset, is rarely complete except in advanced cases, and affects the hair in other areas coincidently with that of the scalp. Again, cretins are not, as a rule, bald. Alopecia is not a constant accompaniment of the various types of thyroid dysfunction or hyperactivity, and when it does occur it almost invariably assumes the characteristics of alopecia areata. The observations recorded in the present paper show that the action of thallium is not interfered with by the simultaneous administration of thyroid extract.

Parathyroid insufficiency, if marked, would be evidenced by tetanoid symptoms, but no such phenomena are observed during the therapeutic action of thallium. The most obvious function of the parathyroid glands is that of regulating certain phases of calcium metabolism, and it is almost certain that some alteration of the serum calcium content is a constant accompaniment of parathyroid dysfunction. Marked lowering of the serum calcium level may occur in rickets, while in osteitis fibrosa associated with adenomata of the parathyroid glands the serum calcium is very high. Alopecia is not, however, a clinical characteristic of either of these/

these conditions, nor does it result from prolonged administration of parathyroid extract (42). The depilatory action of thallium is uninfluenced by the administration of large doses of calcium chloride. There does not, therefore, appear to be any direct association between parathyroid dysfunction and thallium alopecia, and the same may be said of derangements of calcium metabolism, and of alterations in the serum calcium level.

Any effect of thallium on the hair through its intermediate action on the adrenals would be closely linked up with changes in the activity of the sympathetic nervous system, and, as will be mentioned later, stimulation of the sympathetic is also postulated as the cause of thallium alopecia. Hypofunction of either the adrenal or thyroid glands does not, however, occur in conjunction with sympathetic stimulation, and alopecia is not associated with the lowered secretory activity of the adrenals in Addison's disease. Conversely, repeated injections of adrenalin over prolonged periods do not lead to a generalized loss of hair in animals. At most such injections may cause a localized area of alopecia, due to trauma, at the site/

site of injection, if this is always performed in the same place. Aramaky (40) found that injections of adrenalin tended, if anything, to retard the fall of hair due to thallium in animals, and Berblinger (43) states that hyperactivity of the adrenal glands is associated with hirsutism.

As an alternative to the endocrine theory, and closely related to it, is that which supposes thallium to stimulate the sympathetic nervous system, and so cause alopecia (34, 44,45). This theory has been evolved in conjunction with that of endocrine dysfunction to explain the selective action of therapeutic doses of thallium on the scalp hair in humans and on the hair on certain regions in animals. It is supposed that the scalp hairs are supplied by the sympathetic nervous system, whereas the eyebrows, eyelashes and moustache hairs are under the control of the central nervous system and possess no sympathetic innervation. All available data are contradictory to such an hypothesis. The selective action of thallium for certain areas is more apparent than real, as cases are recorded in which total alopecia has occurred, involving the eyebrows, beard, axillae and pubis (46). Case 19 also demonstrates/

demonstrates that the action of thallium is not confined to the scalp hairs alone, as in this case the coarse hairs of a naevus on the cheek were affected by the drug. It is also more than likely that sympathetic vaso-motor nerves normally accompany the blood vessels to all hair-bulbs. Furthermore, hair growth and nutrition are apparently quite independent of nervous influences, since skin grafts containing hairs maintain their vitality provided an adequate blood supply is established. The contained hairs remain intact during the period before nerve regeneration has had time to take place (47,48). In this connection Wright and Harkins (49) report that complete section of the nerves to the scalp, including destruction of the sympathetic fibres, is without effect on hair growth.

Balbi (26) has carried out direct biological and pharmacological investigations to ascertain the state of the endocrine glands and sympathetic nervous system after thallium administration, but was unable to discover any departure from the normal. The results of my own studies of the basal/

basal metabolic rate after thallium have already been discussed, and throw no light on the problem.

Pardo-Castello[27] suggests a clinical and pathological identity between alopecia areata and the alopecia produced by thallium. A clinical comparison of the two types of alopecia reveals two important differences. In the first place the mode of onset in each case is typical. Alopecia areata involving the entire scalp is complete from the commencement in very exceptional cases only. It generally develops slowly as a result of the coalescence of isolated plaques, and follows no definite course. Secondly, the microscopic appearances of thallium hairs and those of alopecia areata are quite distinctive. The chief point of difference is the type of pigment alteration present. On the other hand, Sabouraud (50) has pointed out that sub-epilating doses of X-rays can cause hair deformities which exactly resemble those presented by exclamation-mark hairs. In this case the action is obviously a local one, and does not involve the intervention of the endocrine glands. It is, therefore, unjustifiable to attempt to identify the/

the mode of action of thallium with the hypothetical mechanisms suggested for the pathogenesis of alopecia areata, or vice versa, on the grounds of a clinical resemblance between the two types of alopecia. Even if the two conditions were clinically identical, it would not constitute an argument in favour of the vegetative nervous system or the endocrine glands being involved in the production of thallium alopecia, since recent work (49, 51) does not support the theory that either of these systems is concerned in the pathogenesis of alopecia areata.

From the foregoing it is obvious that there is, at present, scanty evidence in favour of an intermediate involvement of the endocrine glands or sympathetic nervous system being directly responsible for the production of thallium alopecia.

Microscopic examination of the hair and hair-follicles after thallium administration does not give any indication as to the mode of action of the drug. Atrophic and degenerative changes have been observed in the cells of the follicle and bulb (25,36, 38, 52, 53), but are only those which would be expected to accompany a fall of hair such as is produced. The pigment distribution in the hair root/

root and shaft does not show any striking change, a fact which has already been commented on, and which, if anything, argues against the occurrence of any endocrine interference. Thallium has not so far been recovered from hairs which have been shed as a result of its administration. The quantity of thallium which could occur in such hairs is so minute and the methods for ascertaining its presence so imperfect that this cannot as yet be taken as evidence against a direct local action. On the contrary, Sabouraud (20) has found that repeated inunction of an ointment containing small quantities of thallium will cause a local atrophy of hair, although complete alopecia does not occur. Thallium, therefore, seems to possess at least some local inhibitory effect on hair growth.

It is a striking fact that growth power persists in the hair-bulbs for some ten or eleven days after the ingestion of thallium, and subsequently undergoes a period of temporary inhibition. This resemblance between the actions of thallium and X-rays on hair growth favours the conception that thallium/

thallium acts locally, and further suggests that the action is produced in the same type of cell as is that of X-rays. X-rays are distinctly selective, having their maximum effect on young, actively proliferating cells. Should the inhibitory effect of thallium on hair-growth be due to a local action, it seems probable that the more primitive cells of the bulb, possessing considerable growth power, would be most sensitive to it. Such an hypothesis offers an explanation for the susceptibility to the action of thallium displayed by the scalp hair in children. The cells of the follicles and bulb are more active and proliferate more rapidly in this than in other areas, and on that account they may be more sensitive to the action of thallium than those in regions where the power of growth is less.

The theory of the selective action of thallium on the more fully developed and rapidly growing hairs is borne out by case 19 previously described, in which the coarse hairs of a naevus were acted on by the drug while the lanugo hairs situated on the same area were apparently unaffected.

The/

The fact that previously straight hairs may grow in again after thallium epilation in the form of curls also points to some direct action of thallium on the cells of the hair matrix.

(c) /

(c) Favus.

Favus of the scalp, once a prevalent disease in Edinburgh and district, is now rarely seen. Only 11 cases have been treated in the Skin Department of the Royal Infirmary since 1927, whereas from 1908 to 1912 170 cases were dealt with.

2./

2. Tinea Corporis.

(a) Tinea circinata. (Fig. 8).

Tinea circinata was recognised as a clinical entity by Willan and Bateman and was depicted in their Atlas long before its mycotic origin was discovered. Plumbe (54) also describes this disease. For some years the prevailing opinions as to the exact delimitation of T. circinata were far from unanimous, and it was frequently confused with Seborrhoea corporis, eczema and p. rosea. Cazenave (55) redescribed the condition in 1850, and drew attention to the similar identity of T. capitis. Bazin (56) was the first to discover fungus in T. circinata lesions. Although the position should have been clarified by these publications considerable doubt shrouded the subject for some time afterwards, and as late as 1895 Hebra and Kaposi (57) asserted that P. rosea, described by Gibert in 1860 (58) was identical with T. circinata. Such errors in diagnosis do not now arise and T. circinata is now one of the most easily recognised skin diseases.

Apart/

Fig. 8. Tinea circinata.

Apart from the biological deductions of Jadassolm which will be discussed later (see section on Immunity to Fungus Infections, p. 113), no advances have been made in recent years in the study of microsporon or trichophyton infections of the glabrous skin presenting the clinical features of Tinea circinata. It has gradually become recognised however that these organisms, along with epidermophyton, may produce a different type of lesion which bears little resemblance to Tinea circinata. Such infections are grouped together and classified as Epidermomycosis.

(b) Epidermomycosis. /

(b) Epidermomycosis.

From the etymological standpoint the term epidermomycosis includes all mycotic infections of the glabrous skin as distinct from those involving the hair and nails. The term as used currently indicates a very restricted though frequently met with group of superficial mycotic skin infections which are essentially intertriginous in character or are extensions of lesions situated in the body folds. Thus defined it does not refer to Tinea circinata, Tinea versicolor, Tinea imbricata, erythrasma or favus. Epidermomycotic eruptions are produced in most cases by species of the genus trichophyton, epidermophyton or microsporum, and only these infections will be considered. Budding organisms of the genus cryptococcus, monilia, and saccharomyces, may also cause somewhat similar lesions, but these are considered separately under the terms Saccharomycosis epidermica, Cryptococcosis epidermica and Blastomycosis epidermica. These organisms and the diseases which they produce will not be dealt with here.

On account of the multiplicity of fungi which are capable of producing the eruptions of this group, the terms epidermophytosis and trichophytosis are unsuitable, since they suggest a specific etiological/

etiological agent; moreover morphologically identical lesions can be caused by different species and there is no constant relationship between the species of infecting fungus and the type of lesion produced.

The history of the evolution of the disease and its separation from other disease entities is of great interest. Devergie(59) in 1857 was the first to recognise and describe a trichophyton infection of the groin, and thereby extend the conception of mycotic infections of the glabrous skin beyond the narrow limits of Tinea circinata. In 1860 Hebra (60) gave an accurate clinical description of epidermomycosis of the groin under the name of eczema marginatum, but he did not recognise the mycotic etiology of the condition. In 1864 Köbner(61) demonstrated the presence of mycelium in the lesions of Eczema marginatum, and this was confirmed by Pick in 1869 (62). Both these authors performed successful inoculation experiments with material from the lesions. Hebra then admitted the presence of mycelium in Eczema marginatum (63), but did not recognise the condition as a mycotic infection. A small epidemic of inguinal ringworm was/

was then described by Fox in 1878 (64). Kaposi (65) refused to admit the etiological role of the fungus present in the lesions, because the clinical appearance of the lesions was so dissimilar to that of Tinea circinata. On the other hand Besnier and Doyen in their translation of Kaposi's book add notes to the effect that they considered the condition to be a fungus infection, and they further question the propriety of the term eczema marginatum.

The work of the foregoing authors was apparently not generally recognised, and subsequent literature contains records of cases of Eczema marginatum or inguinal trochophytosis under the title of seborrhoeic eczema. Sabouraud commenced to study the subject in 1895. He isolated an epidermophyton from the lesions and during the succeeding years came to the conclusion that the parasite was always the same, and that the lesions which it produced were always identical. By 1910 (Les Teignes) he referred to Eczema marginatum as inguinal epidermophytosis which he considered to be a specific mycotic infection, the causative fungus/

fungus differing from trichophyton in its predilection for the body folds, in the naked eye and microscopic appearances of its cultures, and in the type of lesion which it produced. This became the generally accepted view of the condition, which was therefore removed from the category Eczema and placed with the superficial mycotic diseases. Sabouraud criticises the title eczema marginatum given to the disease by Hebra in the following caustic phrase: "Eczema était fâcheux, marginatum était meilleur." Up to that time inguinal epidermomycosis does not appear to have been a very prevalent disease.

Coincident with the slow emergence of mycotic infections of the groin from the eczema class, parallel observations were being made in regard to certain eruptions of the feet and hands. Tilbury Fox (66) and Pellizari (67) both reported cases of mycotic infections of the palms and soles secondary to a co-existing Tinea circinata or capitis. Djelaleddin-Mouktar (68) in 1892 was the first to demonstrate primary mycotic infection of the soles, in a series of 25 cases. In 1908 Whitfield (69) published a report of six cases in which/

which he found fungus microscopically. In 1910 Sabouraud (70) described dyshydrotic and macerated intertriginous lesions of the fingers and toes from which he isolated Epidermophyton inguinale. He drew attention to the association of such lesions with inguinal epidermophytosis, and observed that the interdigital spaces of the feet, and especially the fourth interdigital spaces, were more frequently affected than the hands. Sabouraud (70) also reported the finding of T. crateriforme, T. acuminatum, T. violaceum, T. persicolor, and M. audouini respectively in five cases of scaly or vesicular eruptions of the palms and soles. Whitfield (71) in 1911 described 15 cases of hand and foot eruptions in which fungus was found. From this series and his previously reported cases he divides eruptions of the hands and feet which are associated with mycotic infection into an acute vesiculobullous or hyshydrotic variety, a chronic intertriginous form, and a chronic hyperkeratotic form. Sabouraud in 1911 considered that mycotic infection of/

of the groins and feet belonged to one or other of the following categories:-

(1) Groin lesions: those due to E. inguinale and those due to E. rubrum.

(2) Hyperkeratotic plantar and palmar lesions due to T. violaceum, T. acuminatum, and E. inguinale.

(3) Toe and finger lesions due to E. inguinale.

(4) Kerion lesions of the backs of the hands.

He does not appear to include the scaly and vesicular types reported in "Les Teignes" in this suggested clinical division.

In 1914 Kauffman-Wolff (72) demonstrated fungi in dyshydrotic lesions of the hands and feet, and cultivated T. interdigitale, E. cruris, M. lanosum, and T. gypseum respectively in several cases. Kaufman-Wolff was of the opinion that about 30 per cent. of such lesions were mycotic in origin. During the succeeding years reports were published by different authors of cases of dyshydrotic, scaly, intertriginous and hyperkeratotic eruptions of the hands and feet from which various species of fungi were isolated. Of the larger series of cases published may be mentioned those of Ormsby and Mitchell (73), Darier (74) and Williams (75). Ormsby and Mitchell demonstrated fungus in a series of 65 patients suffering from hand and foot eruptions/

eruptions. They obtained a culture of E. inguinale in 6 out of 17 cases in which culture was attempted. Darier examined a series of vesicular eruptions of the hands, and demonstrated fungi in many cases. He was of the opinion that all cases of dyshydrosis except those of venenate origin were due to mycotic infection. Williams investigated a series of eruptions situated on the hands and feet in regard to their possible mycotic origin. He divided plantar eruptions into (1) maceration and fissuring of the interdigital spaces, (2) a deep seated circinate vesicular eruption occurring on the soles and (3) callous formation on the soles. In the 36 cases of the first type fungi were demonstrated microscopically in 13, and five cultures were obtained. These were: Epidermophyton 2 cases, T. lacticolor, T. acuminatum, and T. plicatile 1 case each. In the case of the hand lesions two main types were observed, a macerated intertriginous form, and a dyshydrotic form. T. violaceum, T. amethysticum, and T. asteriodes were isolated from palmar vesicles in 3 cases. Williams is of the opinion that all dyshydrotic eruptions are due to mycotic infection.

In/

In 1927 Weideman (10) tabulated the existing reports from 1910-1926 on the isolation and identification of fungi in cases of epidermomycosis. He found that no less than 21 different species had been isolated from such lesions exclusive of yeast-like organisms. Tr. interdigitale and Tr. rubrum were the most common, in contradistinction to the original view that E. cruris was that most frequently seen. The following table gives the reported species distribution for America, Britain, and Japan.

Table 6.

America (total 272 cases).

Tr. interdigitale	140	Tr. acuminatum	2
E. cruris	54	Tr. granulosum	2
Unidentified	20	Tr. lacticolor	2
Tr. rubrum	17	Tr. plicatile	1
Tr. gypseum	17	Tr. amethysticum	1
O. albicans	8	Tr. violaceum	1
Tr. asteriodes	3	Sp. schenkii	1
Tr. pedis	5		

Britain (total 43 cases).

Tr. cruris	29
Tr. crateriforme	6
Various	8

Japan (total 117 cases)

E. rubrum	66
E. interdigitale	24
Various	27

Gould and Carter (76) state that Tr. interdigitale, Tr. purpureum and Tr. gypseum are the commonest fungi infecting the toes in America.

It would appear that each species is cosmopolitan, but that, as with other types of mycotic infection, certain species predominate in certain geographical regions. With the increasing recognition and incidence of epidermomycosis several broad morphological types have been distinguished. The following clinical descriptions taken from various authors indicate the clinical groups into which cases of epidermomycosis can be separated. As has already been mentioned there does not appear to be any connection between the causative fungus and the type of lesion produced.

Clinical Manifestations of Epidermomycosis.

The eruption may be primarily vesicular, papular, scaly or hyperkeratotic. As a result of irritation and trauma it may become inflamed, fissured and macerated. Its onset may be acute or/

or insidious, and its course is almost always a long one, interspersed with frequent remissions and relapses. A description of the eruption as it occurs on those parts of the body for which it has a predeliction gives a more useful clinical picture than one which has the predominating lesion for its basis.

Epidermomycosis of the feet - This may occur in three forms: (1) scaly.
(2) vesicular.
(3) hyperkeratotic.

It is usually a combination of at least two of these types.

The scaly form commences as a rule between the toes, affecting most commonly the fourth digital space. The scaling may be slight, affecting only the depth of the fold, or it may be abundant, forming a whitish sodden mass of epithelium, which when removed leaves a bright red slightly moist surface with a well defined margin. Small thin walled vesicles may be situated on the borders of the toes. Scaling and vesiculation may also extend/

extend into the fold underneath the toes, and be prolonged on to the sole of the foot as far as the heads of the meta-tarsals. In this situation the edge of the lesion is well defined, and is usually marked by a raised epithelial scale which is attached at the periphery and the free edge of which points towards the centre of the lesion.

The purely vesicular form occurs on the sole of the foot, usually towards the inner aspect in the neighbourhood of the instep. The eruption commences as several isolated vesicles deeply seated in the epidermis. These increase in number, coalesce, and the overlying layer of epidermis is cast off leaving a red oozing surface bordered by a rim of raised epidermis. Surrounding the lesion are numerous closely set vesicles which may also coalesce, or which may remain discrete, the contents drying up to form brownish scales. Small brown dessicated vesicles are characteristic of the disease.

In the hyperkeratotic type thick horny masses form on the soles, and this process is usually accompanied by the formation of painful fissures.

The/

The lesions on the hands closely resemble those which occur on the feet. Interdigital scaling is less marked, and the eruption is usually vesicular, the vesicles being situated on the sides of the fingers and resembling sago grains in appearance. The vesicular eruption may occur on the palms or backs of the hands and in these situations bears a close resemblance to the vesicular form which occurs on the feet. On both hands and feet the eruption may have a very acute onset, and this is specially liable to occur in hot weather.

In 1857 Devergie had observed and described a variety of Tinea corporis affecting the upper and inner aspect of the thighs which was undoubtedly the condition now classed as epidermomycosis or dhobic itch. In 1860 Hebra called attention to the same condition under the name of "eczema marginatum". Hebra's original description of the clinical features and course of the disease is complete in every detail. He described the condition as differing from other forms of eczema in its constant localisation/

localisation on the internal aspect of the thighs, the pubis and the gluteal regions; in its centrifugal spread; in its clearly defined margin; and by its special predilection for males. The disease usually commences on the internal aspect of the thigh in the neighbourhood of the groin as a red plaque with a slightly raised papulo-vesicular border. The centre of the plaque soon becomes less inflamed and takes on a dull reddish brown appearance. The plaque enlarges in size by peripheral extension, and smaller satellite lesions may develop near the spreading margin. In view of Jadassohn's recent observations on immune biological processes in the skin, it is interesting to note that Hebra had already made the observation that when lesions which spread by peripheral extension happen to coalesce, the effects which they produce disappear as soon as they touch one another. The coalescence of such outlying lesions with the main lesions gives the edge of the whole lesion a serpiginous outline. The eruption does not as a rule affect the depth of the groin fold. As a result of heat and moisture the affected area may become/

become macerated, and incessant scratching may produce lichenification. The disease is usually bilateral (66 per cent. of 192 cases were bilateral; White (77)). Sabouraud (78) pointed out the frequent association of epidermomycosis of the upper part of the thighs and a similar infection of the feet, especially of the interdigital clefts.

From the thighs the disease may spread upwards on to the abdomen, and affect the umbilicus where a raw scaly dermatitis with well defined margins is produced. It may be transmitted to the axillae where the lesions closely resemble those on the thighs. The infection may spread posteriorly to involve the permeum and anal fold, where the lesions take on a macerated appearance and may become fissured. Involvement of the scrotum and penis may occur in the form of a papulo vesicular eruption which tends to produce a raw glazed appearance as a result of coalescence of the lesions. White (79) from an analysis of his cases gives the relative frequency with which the above sites are affected as follows: Feet 675 cases, hands 590 cases, scrotum, groin and perineum 567 cases, axillae 179 cases, other areas 27 cases. In some cases more

than/

than one area was affected. The vesicular type was present alone in 24.5 per cent. of cases and the scaly type in 23.1 per cent., the hyperkeratotic type in 6.6 per cent. No age is exempt. For some reason males are approximately twice as prone to the condition as females. There does not appear to be any association between occupation and liability to epidermomycosis. The source of infection is usually some inanimate object, such as clothing, or floors. Direct infection is not common, and in large series of cases such as that of White (1013 cases) familial infection is rare. The disease is essentially chronic and may last intermittently for a large number of years, becoming quiescent in winter, and recurring more or less acutely in warm weather.

The diagnosis of this condition rests on the finding of fungus in material obtained from the lesions. Success in this direction depends largely on a knowledge of which portion of the lesion to examine. The roofs of recent vesicles or old dried vesicles situated at the margin of the affected area are most likely to contain the fungus. Macerated epidermis often yields negative results, and secondary scaling/

scaling due to scratching or excessive treatment must be avoided. Numerous examinations of material from lesions which are not undergoing treatment may be necessary before a diagnosis is reached. In presence of an eruption which suggests epidermomycosis clinically negative microscopic findings, even when repeated, are always unsatisfactory. It is frequently impossible to demonstrate fungus in material from lesions of the hands, the clinical appearance of which suggests a fungus infection, and which may closely resemble lesions on the feet in which fungus can be demonstrated with ease. The clinical significance of such hand eruptions will be discussed later. It is most unlikely that cultures made from material in which no fungus elements have been found microscopically will yield positive results. The reverse is usually the case, namely that culture of the fungus frequently fails in cases where mycelium has been found in scales (see previous table). The various errors in the recognition of fungus microscopically will be discussed later.

Incidence/

Incidence of Epidermomycosis.

C.J. White (82) published a series of 1013 cases of epidermomycosis seen by him between 1910 and 1925. The following table shows the yearly incidence of his cases.

Table 7.

1910 -	3	1914 -	20	1918 -	31	1922 -	118
1911	5	1915	17	1919	79	1923	148
1912	11	1916	21	1920	87	1924	156
1913	14	1917	25	1921	131	1925	147

Better diagnosis

It is thus evident that the incidence of epidermomycosis has greatly increased since 1910. Further this increase is real since the disease was a well recognised one by 1915, and any subsequent increase cannot be attributed to better diagnosis in the light of a fuller knowledge of the condition. Since 1920 it has become very prevalent in America. In 1924 Butler, Houghton and Cooper (80) found mycotic infections of the hands and feet in 13.2 per cent. of 500 men examined. In 1929 Sharp (81) found clinical evidence of epidermomycosis in 517 out of 723 men of the same class as that reported by Butler, Houghton and Cooper. In 1925 Hulsey and Jordan (82) found clinical evidence of mycotic infection of the toes in 67 per cent. of university students. This

was/

was confirmed microscopically in 49 per cent. and cultures were obtained in 5 per cent. Sharp and Taylor (83) reported similar observations. Legge, Bonar and Brown, in 1929, found clinical evidence (84) of epidermomycosis of the feet in 52 per cent. of men and 15 per cent. of women in an examination of 3105 entrants to the University of California. Greenwood and Rockwood (85) examined the feet of 100 diabetic patients and found that in 70 per cent. there was clinical evidence of mycotic infection. In 71 per cent. of those presenting suggestive lesions fungus was found microscopically, but only 13 per cent. yielded pathogenic fungi on culture.

Although mycotic infection of the nails is strictly speaking not included in the group of epidermomycosis, several authors mention the fairly frequent association of the two conditions on the feet. Sabouraud (70) considered ringworm of the nails to be a rare condition, as it occurred only once in his analysed series of 500 cases of mycotic infections, when T. violaceum was isolated. White in 1927 encountered Tinea unguem twenty-three times in his series of 1013 cases of epidermomycosis. Karrenberg/

Karrenberg (86)considered it to be a rare form of fungus infection. On the other hand Johns (87) in 1929 reported the finding of fungus in the toe nails of almost everyone examined in New Orleans. The type of fungus found was not specified. Greenwood and Rockwood (88) found Tinea unguens present in 24 of their 100 diabetic patients and of these 24 cases 70 per cent. showed actively growing mycelium. Williams (88) suggests that an unsuspected nail infection may act as a source of re-infection of the feet, and reports three illustrative cases. Rockwood (89) examined 44 cases of onychia (thick, yellowish, opaque nails) and found fungus microscopically in 64 per cent. of cases. In culture 7 cases yielded penecillium, 7 aspergillus, 3 yeasts, 1 epidermophyton, 1 torula, 1 scopulariospsis, 3 unidentified.

Pathogenic fungi have been isolated by two observers from apparently normal skins. Williams (75) examined scrapings from 39 normal toes and found T. lacticolor and an epidermophyton in two cases. Cornbleet (90) found E. cruris and M. audouini once each in scrapings from between the toes in 100 normal cases examined. Burgess (91) however/

however, failed to observe or cultivate fungi from between the toes in 100 normal cases.

It must be admitted therefore that pathogenic fungi may rarely inhabit the skin without producing any apparent pathological change, in the same way in which virulent bacteria may lead a saprophytic existence in healthy throats.

(3) /

(3) Amycotic Dermatoses resembling Ringworm.

Many authors who have studied eruptions of the hands and feet from the etiological standpoint are of the opinion that most of the dyshydrotic types are due to fungus infection, in spite of the fact that fungi can only be demonstrated in a proportion of such cases.

The following table showing the results of several authors illustrates the percentage of cases in which fungus could be demonstrated, and in which the fungus was shown to be viable by culture.

Table 8.

Author.	No. of cases	Fungus in Scraping		Cultures	
		No.	%	No.	%
Dold (87)	98	95	96	14	15
Hulsey and Jordan(82)	77	49	63	5	10
White and Greenwood (92)	50	25	50	18	40
Williams (75)	36	13	36	5	14

There is a marked difference in the number of cases in which fungus was found microscopically in scrapings and the number of cultures obtained from such material. This may be due to the cultural methods employed, to the presence of other organisms exerting an anti-biotic influence on the fungus, or to the fact that the fungus seen microscopically was either dead or of very low vitality as a result of the inflammatory reaction and allergic phenomena which its presence on the skin had excited. Failure to obtain cultures from material in which fungus elements have been demonstrated does not suggest that these were saprophytic, since saprophytes are much more easily cultured than are the pathogenic varieties.

White (93) enumerates the possible causes of areas of dermatitis which from their clinical appearance suggest a mycotic infection, but in which hyphae cannot be demonstrated, as follows: (1) non-hyphymycetic fungus, (2) hyphomycetes with changed morphology, (3) bacteria, (4) a toxic or allergic reaction, (5) an ultra-microscopic virus. The/

The second and fourth categories are those which have to be considered from the standpoint of the purely hyphal type of mycotic infection.

It is doubtful if the pathogenic fungi which occur in the lesion they produce as branching hyphae ever change their morphology. Such an explanation has been offered for the very atypical refractile elements seen in scales or the roofs of vesicles taken from lesions presenting a clinical appearance suggestive of a mycotic origin. These bodies are short filaments often with club-like ends which branch and unite to form a mosaic pattern. Such mosaic forms have always been the subject of controversy. They are situated exclusively between the epidermal cells, are irregularly headed, and are often associated with small groups of spore like elements. They are most commonly met with in sodden epidermis. The majority of authors deny their mycotic nature and look on them as artefacts produced by air, soaps, fat droplets, crystals, or epidermal debris lying in the intercellular spaces. The beaded appearance is attributed to the intercellular bridges of the rete cells intersecting the/

the spaces between separated cells. Weideman (10) considers that such bodies are degenerate fungus, and White (93) in his inoculation experiments noted that after several days mosaic forms appeared in the scales taken from lesions which had been experimentally produced by fungus inoculation. Greenwood and Rockwood (85) observed transition forms between typical fungus elements, mosaic, and finally fragmented swollen bodies in scrapings from infected toes. They claimed to have seen degenerate forms in direct continuity with undoubted hyphae.

Mosaic forms are seen in scales cleared with xylene, and it is possible to stain them with giemsa. McKee and Lewis (94) claim to have obtained cultures of pathogenic fungi from scales which showed only mosaic forms microscopically. They were unable to demonstrate such mosaic formation in the scales of seborrhoea, psoriasis, lichen planus, pityriasis rosea, pemphigus, and strips of skin removed with a razor subsequent to thorough inunction of grease. They found that mosaic forms could rarely be found in diseases other than conventional epidermomycosis or epidermomykide lesions of the hands and feet.

The/

The fact remains, however, that with the exception of the experiments of McKee and Lewis, all attempts to culture such elements have failed, so that should they in reality be derived from fungus they are rarely viable when they present such appearances. The non-viability of mosaic forms is illustrated by the following experiments. Mosaic formation was found in the scales obtained from five patients suffering from lesions which were suggestive of mycotic infection. These appearances were photographed and several scales were then implanted on Sabouraud's medium. The detailed case reports and the results of culture were as follows.

Case 1.

H.E., female, presented an interdigital intertrigo of 3 fingers of the right hand. This involved the webs of the fingers and extended on to the sides. The area involved was red and scaly, and was bounded by a distinct margin formed by a colarette of scales. On the right palm there was a red scaly and crusted patch with a very definite edge.

Microscopic/

Microscopic examination of scales from all areas showed beaded hyphae-like structures, some arranged in a mosaic, others in rows. All the elements were short, and were greenish in colour, granular and refractile. The segments were square or pear shaped. (Fig. 9). 20 inoculations were made with material from different parts of the lesions. No growth had developed at the end of 3 months.

Case 2. A.F., a male student, presented an interdigital intertrigo affecting all the toes of both feet. The skin of the webs was moist, white and sodden. Microscopically pear shaped and square segments resembling hyphae, arranged in groups and in mosaic formation , were seen. Nine inoculations were made on Sabouraud's medium, but only a growth of staphylococci was obtained. The cultures were negative for fungus at the end of 3 months.

Case 3. Mrs M. presented an interdigital intertrigo of both feet. The skin between the toes was white and sodden, and fissuring was present. Microscopically club/

club shaped and square segments were seen between the epithelial cells, and overlying them. These segments were arranged for the most part in mosaic formation. The substance of the segments was granular and refractile (Fig. 9). No growth of fungus was obtained on culture after three months from any of the 20 inoculations made.

Case 4. A.T., female, presented a paronychia affecting several fingers. The skin of the nail folds was red and swollen, and the bases of the nails were brown, sodden and cracked. Microscopically, abundant club and pear shaped bodies were seen arranged in mosaic, also spore-like bodies. No definite mycelium was observed. No fungus growth was obtained after culturing for three months.

Case 5. This case is particularly interesting as it is an example of dyshydrotic epidermophytide (vide infra) and also presents mosaic forms microscopically. It further demonstrates the antibiotic influence of B. pyocyaneus on fungus growth (vide supra).

A/

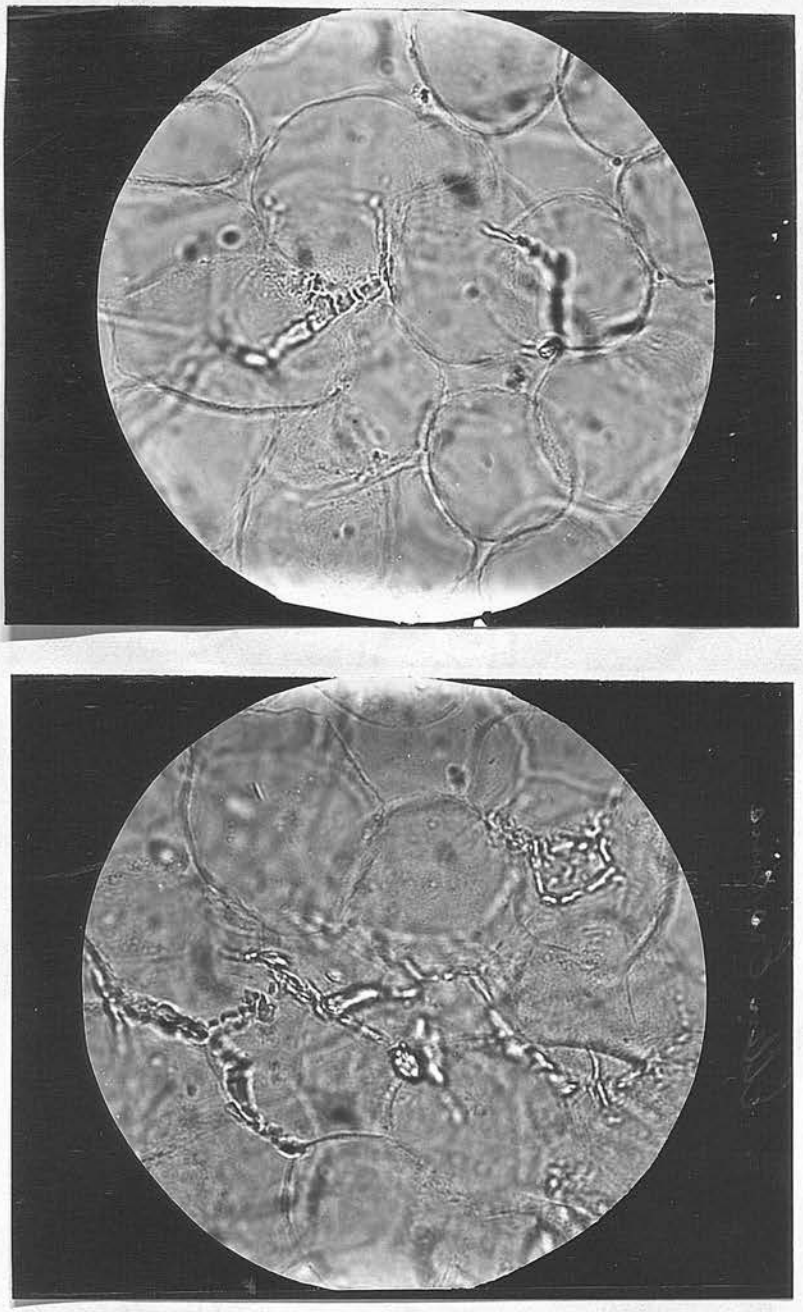
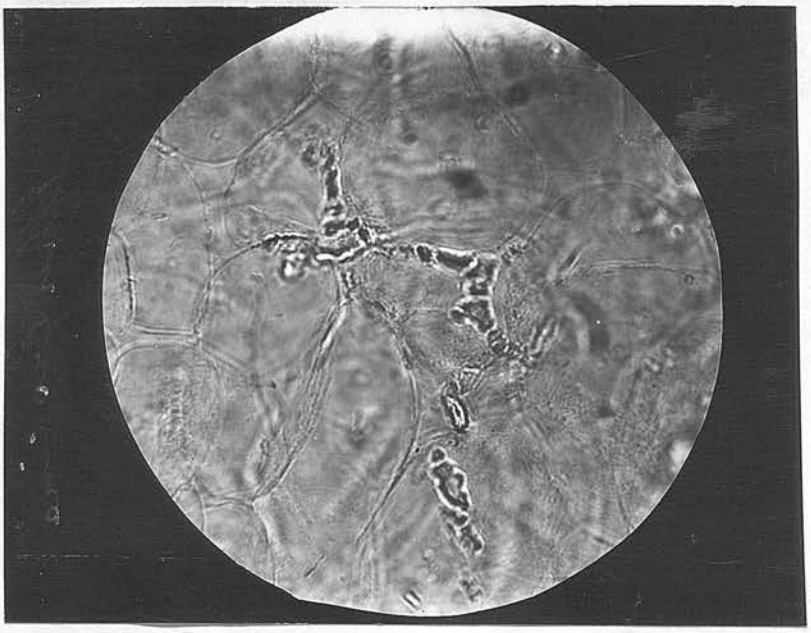


Fig. 9. Case 1. Liquor potassae preparation of scrapings from fingers and palm, showing "mosaic" fungus, pear-shaped bodies, and rectangular segments. (x 400).



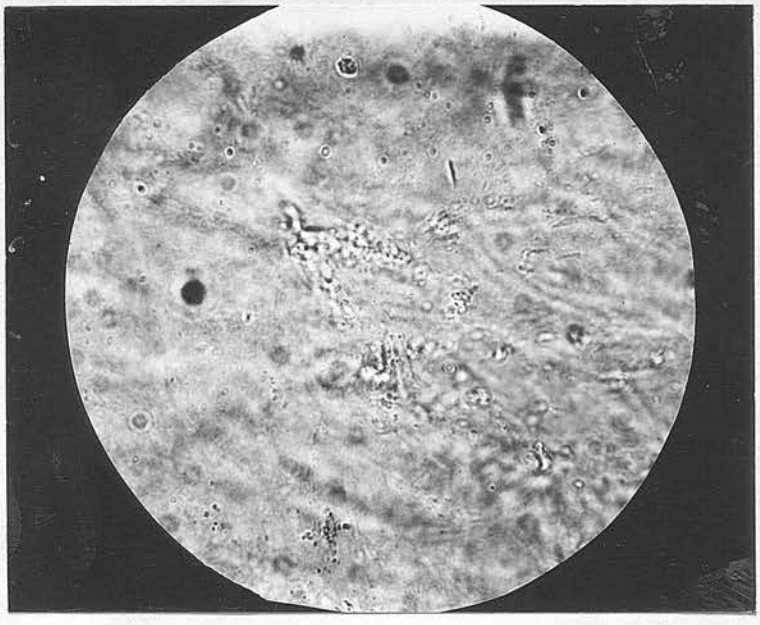


Fig. 9. Case 1. Liquor potassae preparation of scrapings from fingers and palm, showing "mosaic" fungus, pear-shaped bodies, and rectangular segments. (x 400).

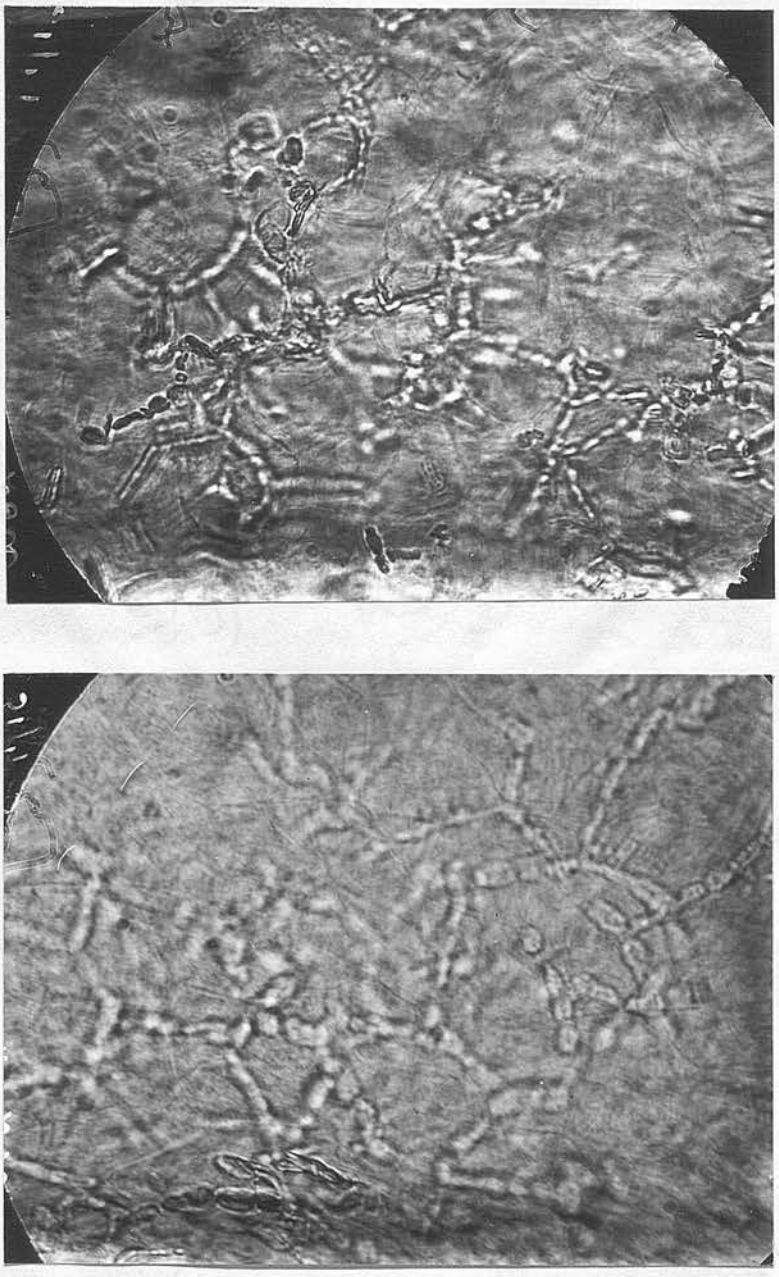


Fig. 9. Case 3. Liquor potassae preparation of scales from toes, showing "mosaic" fungus, (x 400).

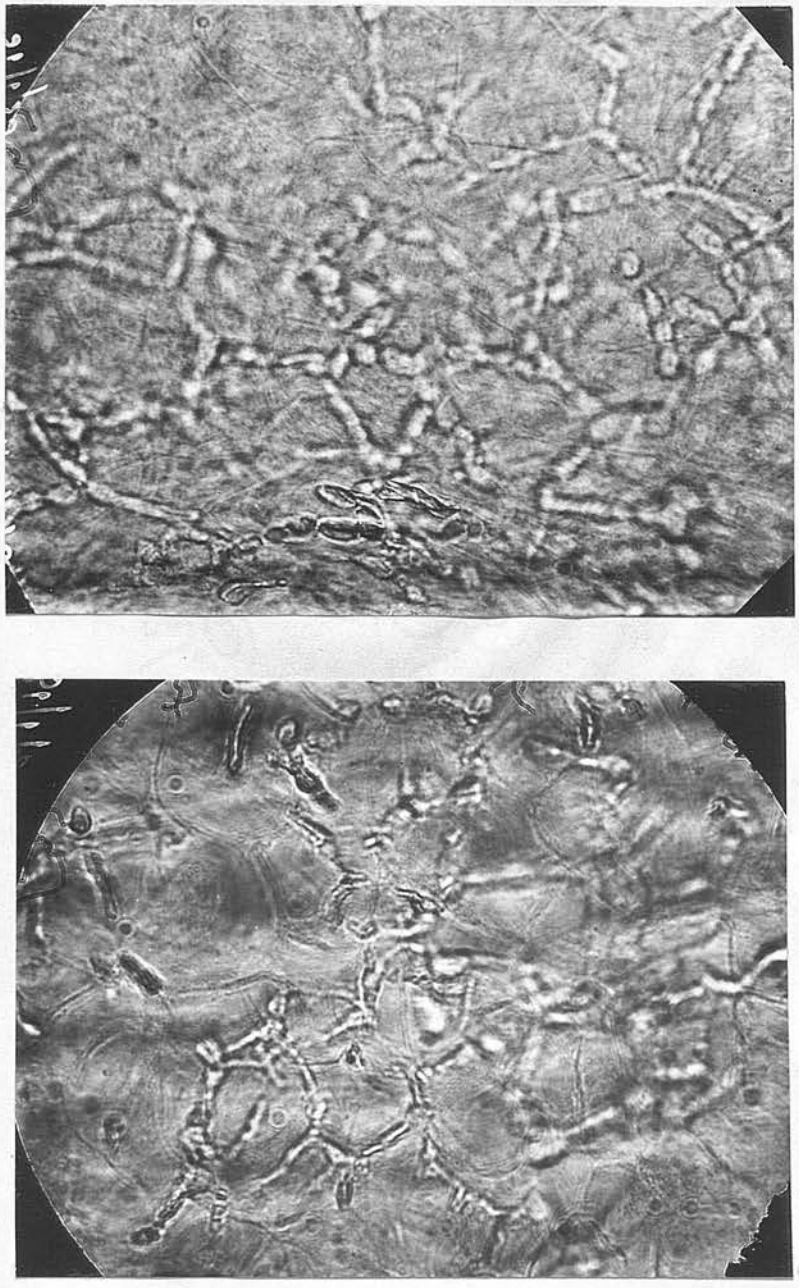


Fig. 9. Case 3. Liquor potassae preparation of scales from toes, showing "mosaic" fungus. (x 400).

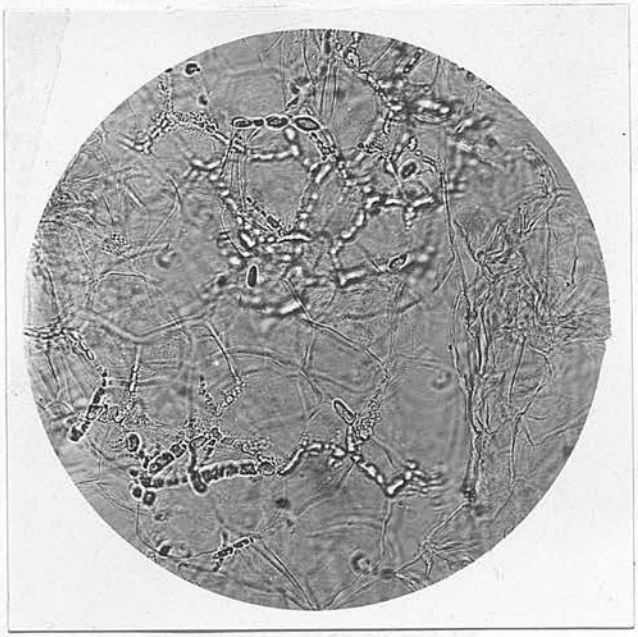


Fig. 9. Case 5.

Liquor potassae preparation of scales from toes showing "mosaic" fungus. (x 300).

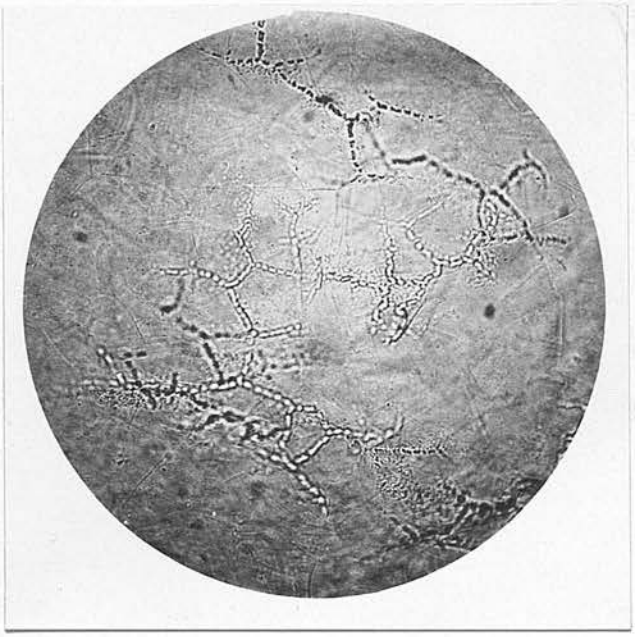


Fig. 9. Case 5.

Liquor potassae preparation of scales from fungus showing "mosaic" fungus. (x 300.)

A married woman aged 33 complained of a patch of scaly dermatitis situated on the inner border of the left foot. This patch had commenced four weeks previously as a small itchy red spot which appeared to have spread as a result of scratching until it attained the dimensions seen on examination. During the period of extension the lesion had always remained dry.

On examination the patch of dermatitis was seen to involve the inner border of the left foot between the heel and the head of the first metatarsal, and to extend slightly on to the sole of the foot in the region of the instep (Fig. 10). The main central portion of this area was dull red, dry and scaly, and was clearly demarcated from the surrounding healthy skin by a marginal colarette of scales. The surface epidermis of this area was tense and thin. There was no induration present. A crop of sago-grain vesicles was situated at both extremities of the central portion of the lesion. The vesicles had a thick roof of brownish epidermis, and were so closely set in places as to be almost confluent. There was no tendency to rupture or oozing of the vesicles/

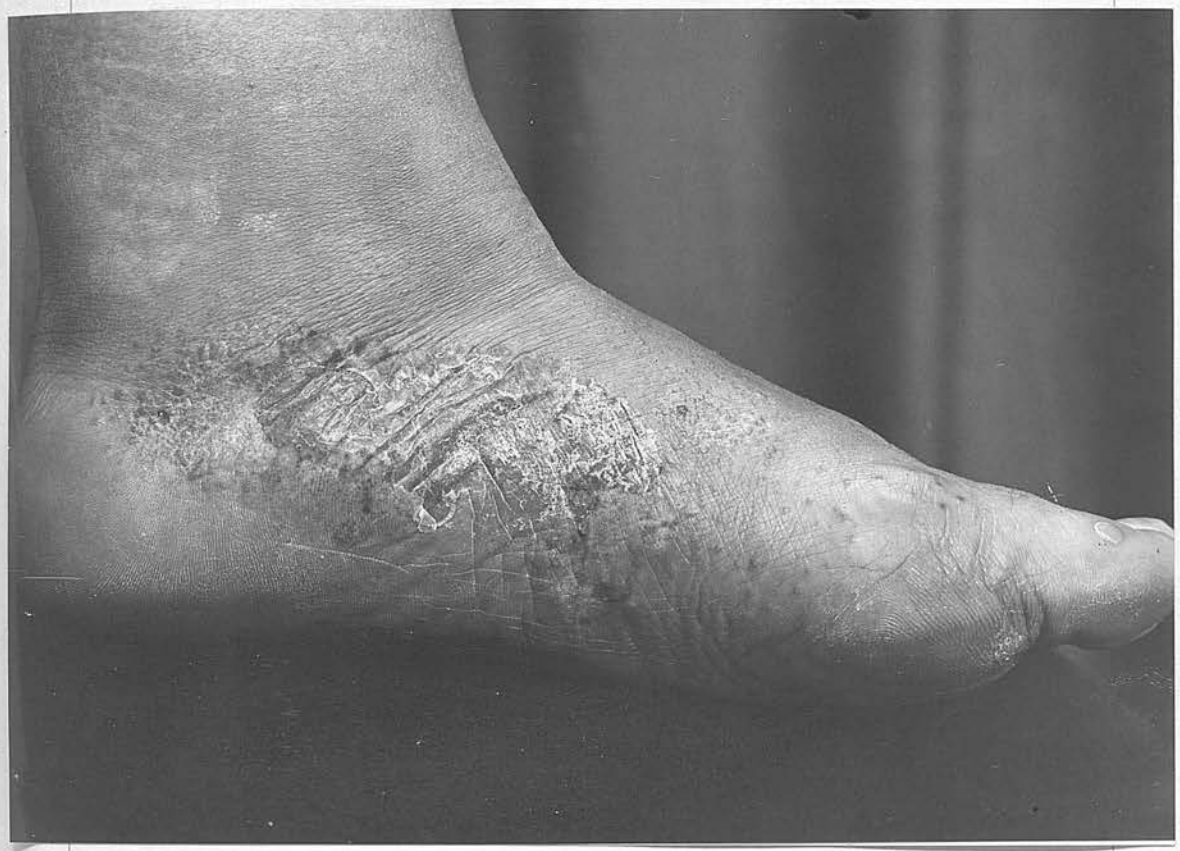


Fig. 10. Case 5.

Epidermophytosis of sole of foot. Typical situation on the inner border and instep.

vesicles, and they apparently dried up spontaneously. This process of dessication involved the roofs of the vesicles which became detached as scales, leaving a thin dull red epidermis visible underneath. The scaly central portion appeared to have been formed in this manner from pre-existing vesicles.

Apart from the area just described the patient was unaware of the existence of any other skin lesions. Further examination, however, revealed the presence of scaling and maceration of the epidermis in the interdigital areas of the toes and in several of these scpaces the skin was fissured and eroded (Fig. 11). On examination of the hands minute sparsely scattered sago-grain like vesicles were discovered on the sides of all the fingers. These vesicles could be felt more easily than seen (Fig. 12). The eruption on the fingers corresponded clinically to that usually described as cheiropompholyx or dyshidrosis. No other skin lesions were found.

On the day on which the patient was examined 0.2 c.c. trichophytine (vide infra) was injected intradermally into the skin of the right forearm and a control injection of 0.2 c.c. maltose broth made/

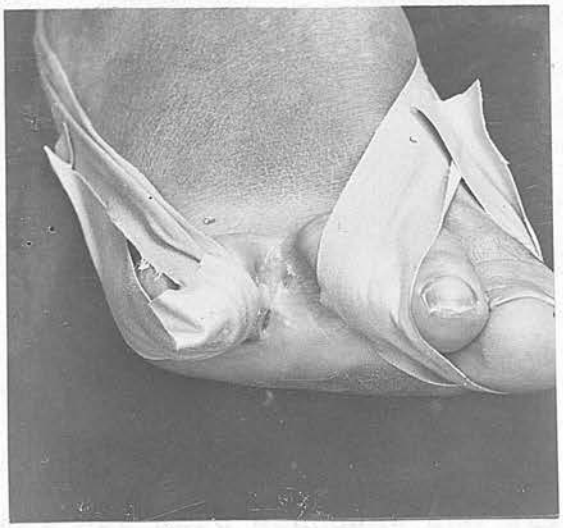


Fig. 11. Interdigital epidermomycosis, showing sodden and macerated scaling between toes.

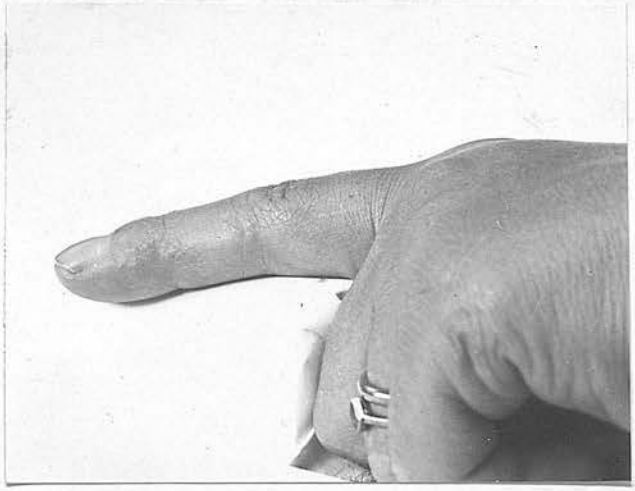


Fig. 12. Commencing "dyshiodrotic" epidermophytide on finger.

made on the left arm. Twenty hours later a bright red slightly oedematous erythema, measuring 4.5 cm. by 3.5 cm., appeared at the site of injection of the trichophytine. The control injection was negative.

Two days later the lesions on the foot and toes were in statuo quo, but the vesicular eruption on the fingers was more abundant and was definitely apparent to the patient on account of considerable itching. The intensity of the trichophytine reaction had in no way abated. On this date (3 days after the first examination) scales were obtained from the feet lesions and examined after treatment with liquor potassae. The roofs of vesicles on the fingers were similarly examined. Preparations from the foot and toes areas showed the presence of fungus elements in abundance. Mosaic forms were found in the scrapings from the hands. Inoculations from the lesions were made on Sabouraud's proof medium, and a sample of the patient's blood was inoculated on maltose broth. The cultures were incubated at 24°C.

The treatment recommended was the application of 1 per cent. iodine in spirit, followed by a 1 per cent. tar paste. Disinfection of clothing was also/

also recommended.

The eruption could not be observed further as the patient was obliged to leave Edinburgh, but she supplied the following information regarding its subsequent course by letter. The trichophytine reaction became larger, and the skin surface involved was red and rough up to the 6th day after its onset when it commenced to fade gradually. The feet lesions had practically healed by the 14th day from the date of the first examination. Fresh crops of blisters had continued to appear on the hands for 10 days, and regressed with a considerable amount of accompanying desquamation.

Results of Cultural Examination. (12 inoculations were made from each lesion on Sabouraud's maltose agar). The blood yielded no culture growth. The inoculated material obtained from between the toes yielded an abundant growth of B. pyocyaneus only. Cultures made from the hand lesions remained sterile. In the inoculations made from the lesions on the side of the foot a white fungus growth appeared on the 4th day, and gradually increased in size. The fungus was identified as Epidermophyton rubrum. In order/

order to ascertain whether the B. pyocyaneous isolated from the interdigital areas might have inhibited the growth of any fungus co-existing with it in the inoculated material, four inoculations of a mixture of B. pyocyaneous and Epidermophyton were made. It was found that the bacillus grew luxuriantly, growth commencing within 24 hours, but no fungus growth appeared and the cultures were eventually discarded on the 40th day.

As regards the source of the infection, the patient may have acquired it when on a visit to South Africa a year previously, although she did not notice any skin eruption at that time. It is also possible that she became infected from her husband who arrived home from Africa six weeks prior to the appearance of the patient's foot lesion. The husband was examined, and the skin between the toes found to be macerated and scaly. Liquor potassae preparations of these scales showed the presence of fungus elements which closely resembled those found in the patient's lesions. No cultures were made from the husband's skin.

The/

The nearest approach to mosaic formation which I have seen presented by a true fungus in a liquor potassae preparation was that in scales obtained from the macerated epithelium in the fourth interdigital space of an Indian's foot. The appearance is shown in Fig. 13 . The elements are regular in contour and size and are obviously made up of the faceted segments of hyphae. There is no possible dubiety with regard to the nature of the elements, which are obviously particulate and the appearances are not due to an optical effect produced by intercellular air or debris. The mosaic appearance is merely suggested and is not complete. Culture yielded Epidermophyton rubrum.

There is ample proof of the correctness of the suggestion that mycotic like eruptions may be toxic or allergic reactions to fungus products derived from a distant primary focus. Mitchell (95) in dealing with the etiology of the superficial scaly type of palmar and plantar eruption stressed the fact that the most careful search may fail to reveal fungi, and is "inclined to believe that fungi which are so few in number as to escape detection by the intensive/

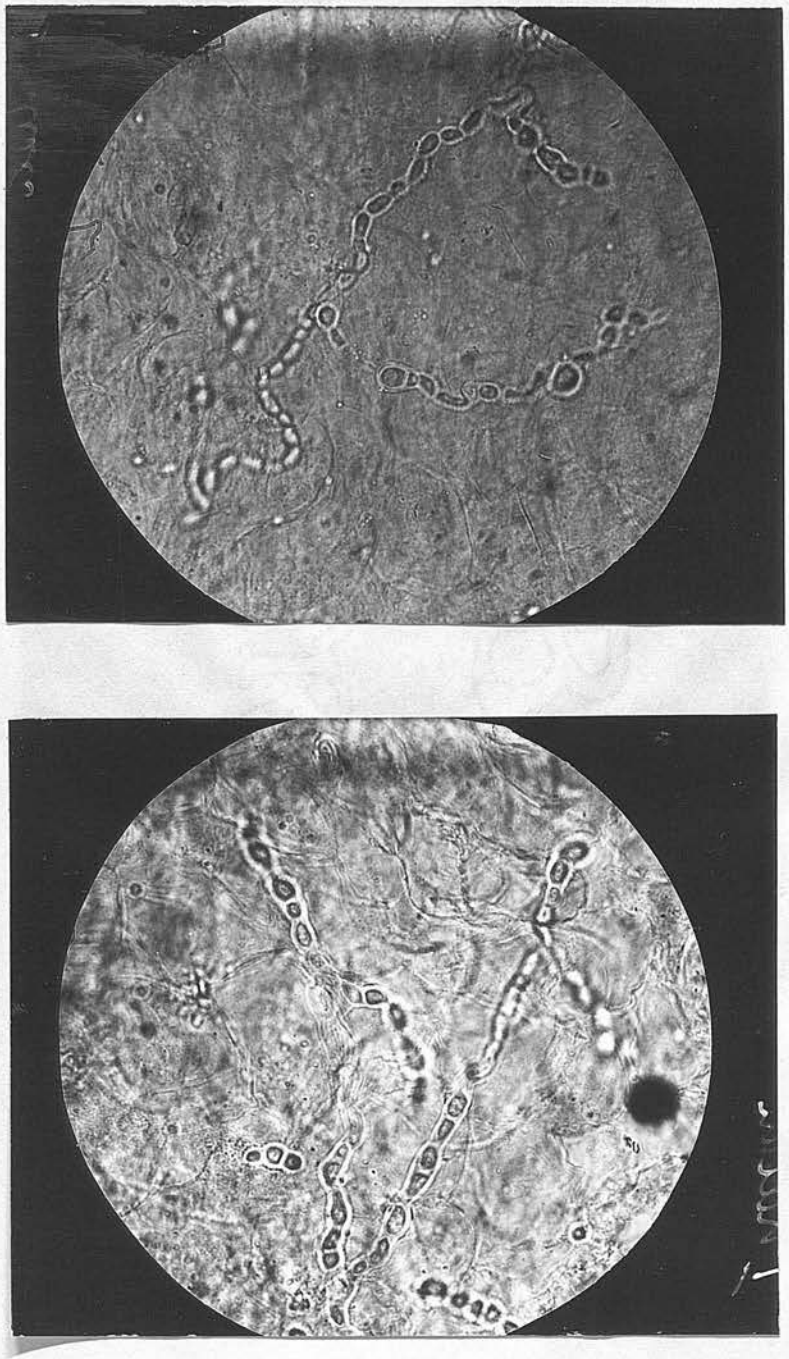


Fig. 13. Liquor potassae preparation of scales from between toes showing E. rubrum tending to take a "mosaic" formation. (x 400).

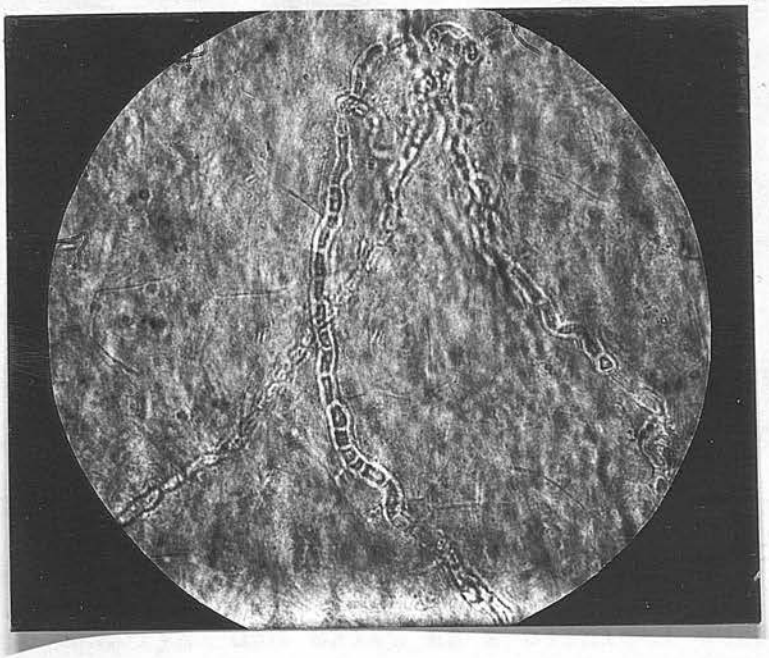


Fig. 13. Liquor potassae preparation of scales from between toes showing E. rubrum tending to take a "mosaic" formation. (x 400).

intensive efforts of an experienced technician are not capable of exciting cutaneous lesions". Lehman (96) in a study of 131 cases presenting dyshydrotic eruptions of the hands and feet found that mycotic infection accounted for 86 cases, chemical irritation for 25, and that no external cause could be discovered for the remaining 20. He therefore regarded the latter class as tru e examples of cheiropompholyx , and was of the opinion that nervous influences were largely responsible for the eruption. Wise and Wile in the discussion on Lehman's paper also upheld the view that cheiropompholyx did exist as a clinical entity and that it was due to some metabolic or nervous disturbance.

The position at present with regard to scaly and dyshydrotic eruptions of the hands and feet is much less certain than it first appeared when the fungus origin of certain of these eruptions was demonstrated. The sweeping assertions which were made at that time have not been justified by subsequent and more detailed observations. While fungus infections as a rule produce lesions which present/

present suggestive characteristics, these are not sufficiently well defined to enable a certain diagnosis to be made in the absence of microscopic examination. Chemical and probably also bacterial irritants can produce exactly similar appearances. If careful and repeated microscopic examination fails to reveal the presence of fungi in the lesions, it is hardly justifiable to consider the eruption to be mycotic in spite of such negative findings. In such circumstances it may still be indirectly due to fungi, being an allergic reaction to fungus products derived from a primary fungus infection situated in some other region. If no such primary focus exists, it is most unlikely that the eruption has any connection with fungus, but is due to some external or internal irritant of an entirely different nature.

(4) Immunity to Fungus Infections.

With the researches of Plato and Neisser (97) in 1902 the subject of mycotic infection of the skin passed from the purely morphological and bacteriological periods into the third or biological period of its evolution. It had long been known that superficial fungus infections of the scalp in children disappeared at or shortly after puberty, thus indicating the spontaneous appearance of some inhibitory factor. It was ~~also~~ recognised and stressed by Sabouraud that deep seated inflammatory infections of the scalp healed much more quickly than the scaly superficial types. It was also observed that in deep seated mycotic infections of the scalp, or in other words kerions, (Fig. 14) a general disturbance, consisting of fever, widespread adenitis, enlargement of the spleen, leucocytosis, and a positive diazo reaction often accompanied the local lesion. This variability in the behaviour of different types of mycotic infections and especially the marked difference between the superficial and deep seated varieties, while undoubtedly depending to some extent on the nature of the infecting fungus/

Fig. 14. Kerion Celsi.

fungus, also suggested the development of varying degrees of tissue immunity towards the infection. Plato and Neisser (97) showed that an extract of Trichophyton fungus produced an inflammatory reaction when injected into the skin of a subject suffering from a deep seated form of ringworm, whereas no such reaction was obtained in normal persons (~~Fig. 12~~). Further observations along these lines were made by Bloch(98), Truffi(99), Bruhns (100),Prytek(101) Lombardo and others(102). The results obtained by these observers show that microsporums, achorions and trichophytons contain a common toxin which is probably a mixture of endo- and exotoxins and can be extracted from cultures of these organisms by a similar method to that used in the preparation of old tuberculin. Bloch, Labouchere and Schaaf (103) have analysed this product, which has been named trichophytine, and find it to be a stable substance. It resists drying and heating (100°C.) and oxidising and reducing agents, but is destroyed by Na_2SO_4.

It/

It is slightly soluble in water, insoluble in organic solvents except glycerine and glycol. It contains N but not S, and does not give the reactions of albumen. It is dialisable. The active substance gives polysaccharide reactions, and the polysaccharide content and the biological activity correspond. This toxin produces a marked inflammatory skin reaction (Fig. 15) on intradermal injection in patients suffering from the deep seated kerion type of ringworm infection, irrespective of the causative fungus, although this type of lesion is most commonly due to a trichophyton of the gypseum group. Uninfected persons or those suffering from superficial trichophyton and microsporon infections either show a very weak reaction or none at all. It has been found recently,however, that the reaction may be positive in inflamed and fissured mycotic infections of the feet and hands (Jadassohn, (104)). Sloimovici and Ullmo (105) have recently applied the trichophytin reaction to a large number of cases/

Fig. 15. 24 hr. old Trichophytine Reaction.

cases, including mycotic infections of the skin, other types of skin lesions, and in cases of visceral tuberculosis. Control injections of tuberculin and bouillon were made. The results obtained entirely confirmed the specificity of the reaction as a group reaction produced by inflammatory types of mycotic infection of the skin. When positive the intensity of the reaction corresponds to the severity of the local lesion, and it develops with the local lesion, reaching its acme at the same time. This hypersensitivity or allergy is developed by the entire skin surface, but Pedderson (106) has shown that it may be most intense in the immediate neighbourhood of the primary lesion. The close correspondence between the development of the hypersensitivity and that of the primary lesion suggests that the two sets of phenomena are closely related to one another or are interdependent, the primary lesion being modified by the allergy which it has produced. Not only is the duration of kerion lesions short, but they rapidly become free of fungus, so that a close relationship/

relationship exists between the degree of allergy present, the degree of inflammation, the quantity of infecting fungus and the duration of the disease. In other words, the greater is the allergy, the more intense is the inflammation, the less numerous are the fungus elements, and the shorter is the course of the disease. While the local lesions heals rapidly, the skin hypersensitivity persists unaltered for years, so that a positive reaction is of limited diagnostic value. The capacity of the skin to give a persistently positive reaction to trichophytine is of importance when compared with the analogous tuberculin and luetin reactions, since it demonstrates that a strongly positive reaction to organismal products is not a sure indication of the presence of an active infection with the corresponding organisms. The hypersensitivity appears to be entirely limited to the skin cells, and the blood and tissue fluids have not been shown to contain any immune bodies.

The hypersensitivity not only regulates the evolution of the local lesion, but by its persistence also/

also confers a complete or partial protection to subsequent mycotic infections, so that these either do not produce lesions, or else the resulting lesion runs a modified course. Superficial mycotic infections do not give rise to any demonstrable hypersensitivity of the skin to trichophytine except in the case of inflamed and fissured mycotic lesions of the feet and hands. Sulzberger has recently shown (107) that in this particular type of superficial mycotic infection not only does the skin of the patient react to the intradermal injection of trichophytine but it also reacts with the formation of an eczematous eruption to the mere application of trichophytine to the intact skin. Normal skins give a negative reaction. This method of applying the test has not so far been used in the deep seated forms of ringworm.

In the case of superficial scalp infections the non-development of allergy is associated with a markedly prolonged course of the disease. The spontaneous disappearance of scalp infections cannot be explained by the development of specific allergy with a subsequent change in the reaction to the infection. It is probably due to alterations in the/

the sebaceous secretions or in the texture of the hair coincident with the normal changes associated with puberty. Even on the scalp, however, a certain degree of immunity appears to develop, since the epidermal infection ceases to spread very shortly after it has become established, although the fungus continues to grow actively in those hairs which are already infected. This point was emphasised by Sabouraud.

Similarly with regard to patches of Tinea circinata occurring on the glabrous skin, Jadassohn (108) suggests that a certain degree of immunity develops in the skin immediately adjacent to the spreading edge of the lesion, and that this local immunity limits the spread of the infection for a time at least. This would explain the failure of attempts to experimentally inoculate the skin adjacent to the edge of a patch of Tinea circinata with the causative fungus, the skin in that area having developed a temporary immunity towards the fungus and failing to react to it. It would also explain the fact that when two ringed patches coalesce the contiguous edges do not invade the opposite lesions, but break to form a kidney shaped or figure of eight lesion/

lesion. On this hypothesis the concentric rings of active disease often seen in large patches of tinea corporis would be explained by supposing that the fungus grew outwards from the edge of the lesion on to an area of skin which had developed immunity to the infection. On this area no lesion would be produced, but immediately the fungus had passed beyond the immune zone the usual papulo-vesicular reaction would take place. This reaction would again be limited by the development of an immunity at its periphery and the same process might be repeated several times, so producing a concentrically ringed lesion.

(5)/

(5) Mycides.

Those mycotic infections which produce allergic changes in the entire skin surface may also be associated with widespread secondary eruptions due to the hematogenous spread of fungus elements derived from the primary focus or to circulating toxins having their origin in this situation. These agents lodge in the skin and there produce their corresponding manifestations. Jadassohn (109) first drew attention to such secondary eruptions in 1912 under the title of lichenoid trichophytide, and Guth (110) supplemented this report shortly afterwards. This is the type of eruption which occurs most commonly in association with a deep seated primary mycotic infection, and many subsequent series of cases have been reported.

The eruption occurs usually at the height of development of the kerion lesion, when this is acutely inflamed, and when fungous elements are difficult to find in it. The eruptions are distributed more or less symmetrically on the trunk and/

and limbs, and may occur on the face. It consists of minute profusely scattered or grouped lichen-like papules, which are rose pink, purplish, or brownish in colour. They are often surmounted by a scale, a crust or a minute pustule. These papules appear in crops which disappear rapidly, and the whole duration of the eruption is from 1-3 weeks. The papules are for the most part situated at the mouths of the hair follicles. Occasionally they are capped by a mass of horny material which forms a small spine. In rare instances the lesions are limited to the neighbourhood of the primary lesion and the eruption has a corymbiform appearance.

Following the recognition of this type of lesion Bloch (111), Pulvermacher (112) and Sutter (113) reported cases of erythema nodosum coincident with kerion lesions. The erythema nodosum type does not differ clinically from erythema nodosum lesions due to other causes. Sutter has described a scarlatiniform variety, which consists of a wide-spread scarlatiniform erythema, associated with congestion of the soft palate. The erythema breaks up into isolated patches, and later lichenoid papules/

papules appear and the exanthem takes on the appearance of a lichenoid trichophytide. Bloch (114) reported an erythema multiforme-like trychophytide, and Bloch (115), Brusgaard (116) and Pulvermacher (112) reported pustular trichophytides, consisting of a symmetrical eruption of small pustules situated on an inflamed base, and distributed on the trunk and limbs.

All these skin manifestations may be accompanied by signs of general disturbance such as fever, rigors, adenitis, enlargement of the spleen, and a leucocytosis.

Williams (117) reported a series of cases of epidermophytosis of the hands and feet, complicated by secondary vesicular and erythematous eruptions on the hands, feet, and arms, in which no mycotic origin could be demonstrated. This was the first observation to suggest that mycides might occur in association with superficial mycotic infections. Beyond the coindicence of the two types of eruption, however, Williams had no proof

that the secondary eruption was dependent on the primary lesion, and he did not demonstrate any skin allergy. Walthard (118) published a case resembling those described by Williams, in which he found the trichophytine reaction to be positive. W. Jadassohn and Peck (119) studied a series of 24 cases of primary epidermomycosis of the feet which also presented secondary sterile vesicular or dyshydrotic lesions of the hands. The trichophytine reaction was strongly positive in all cases, and was frequently followed by an exacerbation of the dyshydrotic eruption. Peck (120) reported a further series of 23 cases presenting similar features. In one case he found fungus elements in the secondary eruption on the hands, and in one case obtained a blood culture of the same epidermophyton as was found in the foot lesions. He was able to reproduce the whole syndrome experimentally in a human subject, by inoculating the interdigital spaces of the feet. These observations prove the correctness of Williams' original supposition, and the production of skin allergy and the appearance of mycide eruptions in association with a superficial primary mycotic/

mycotic infection must be admitted. The term Epidermatophytide has been suggested for this particular type of eruption, in place of trichophytide, in view of the fact that an epidermophyton is responsible for the primary lesion. This type of secondary eruption is structurally an eczematous dermatitis, and it demonstrates that fungus elements or their metabolic products are capable of producing an eczema. In this connection Sulzberger(107) has shown that trichophytine applied as a wet dressing to an intact skin which gives an allergic reaction to the intradermal injection of trichophytine, can produce an eczema eruption at the area of contact.

Williams (121) has recently reported a case of severe epidermomycosis of the groin complicated by a vesicular eruption on the hands and feet. The skin gave a positive reaction both to the contact test with, and the intradermal injection of trichophytine. This he regards as an example of a primary superficial epidermomycosis giving rise to allergic changes in the skin and an epidermophytide eruption/

eruption.

The demonstration by White (122) of fungus elements in the inguinal lymph glands in a case of epidermomycosis of the feet is further proof of the occurrence of deep penetration of fungi from superficial mycotic lesions.

The interpretation of these clinical observations is somewhat difficult, but there is now convincing evidence to show that the lesions referred to are caused by material disseminated from a primary focus of infection and lodging in the skin. The varied types of eruption have several features in common. They all occur during the course of an inflamed or deep seated primary mycotic lesions, and they are constantly associated with a high degree of skin allergy towards trichophytine. In many cases the secondary eruption appears to have been precipitated by the injection of trichophytine, and in the case of the lichenoid and erythema multiforme varieties the reaction to trichophytine has been observed to take the same form as the secondary lesions.

Fungi have been recovered from the blood of patients who were developing trichophytide eruptions/

eruptions by Ambrosoli (123), Jessner(124), Passini(125) Sutter(113), Arzt and Fuhs(126), Masia(127), and Peck(120). A positive blood culture is difficult to obtain, however, and has only been found at the commencement of the eruption. Ambrosoli (quoted from Sulzberger(128)made 1269 blood cultures in 700 cases of trichophytosis, and only secured two positive results. The species of fungus isolated from the blood has as a rule been that commonly associated with deep seated infections, namely Trichophyton gypseum, T.acuminatum, and T. crateriforme, but Epidermophyton, Achorion and even Microsporon audouini have been isolated. The characters shown by the secondary eruption are not dependent on the species of fungus causing the primary lesion, and one species can be associated with different types of secondary eruption in individual cases. In a case reported by Bloch two types of trichophytide occurred simultaneously in the same lesion. As with blood culture very few attempts to demonstrate fungi in the secondary lesions have been successful. Guth(110), Sutter (113) Arzt and Fuhs(126), Ambrosoli(129) and Martinotti (130) have, however, demonstrated fungus elements in this situation. It is unlikely that such fungus reached the lesion from the exterior , since the lesions/

lesions were far removed from the primary focus, and occurred at a time when fungus elements were extremely scanty in, or absent from, the primary lesion, having disappeared as a result of the intense inflammation or the development of local immunity. Moreover the positive blood cultures, although obtained in a very small proportion of cases, show that hematogenous dissemination of fungus elements can occur. Further evidence of the possible deep seated invasion of fungus in the region of kerion lesions and in association with mycotic infection of the feet is presented by the observations of Sutter(113) and White (122) who demonstrated fungi in the lymph glands draining such areas.

Experimental inoculation of animals with various species of fungi has given results which support the theory that trichophytides are due to a hematogenous infection of the skin with fungus elements. In 1916 Saeves (131) succeeded in producing skin lesions on shaved and scarified areas following the intra-cardial injection of fungi. This/

This was confirmed by Kogoz (132) who produced similar lesions by injecting emulsions of spores of Achorion quinckeanum into the testicles, lumbar sac, liver and sub-arachnoid spaces, of guinea pigs. Fried and Segal (133) injected Tr. gypseum intracardially and intravenously into rabbits, and observed scaly lesions and alopecia to develop on lightly scarified areas of skin, after 12-18 days. Scarification appeared to be necessary for the production of the cutaneous lesions. The parasites disappeared rapidly from the blood during the first 28 hours, but could still be found in small quantities up to the 3rd day. Jadassohn (104) found that in animals trichophyton quickly entered the blood after cutaneous inoculation. Sulzberger (128) confirmed this observation, finding that if guinea pigs were infected cutaneously with Achorion quinckeanum, fungus elements could be found in the blood within 24 hours, and that they soon disappeared. On the 10th-13th day they were again abundant, and the second appearance of fungi coincided with the acme of the primary lesion and of the skin allergy to trichophytine.

These results correspond closely with the events which occur in the development of mycides in/

in human beings, as regards the time of appearance of the secondary lesions in relation to the state of development of the primary lesion and of the skin allergy.

From observations on patients and from the results of animal experiments there is no doubt of the direct causal relationship between the primary infection and the secondary lesions. The majority of observers are agreed that fungus elements enter the blood stream, and are conveyed to the skin where they give rise to various types of lesions. The hematogenous spread is accompanied in many cases by a transitory general disturbance with fever. The skin lesions are almost always situated in or about the hair follicles, and the most likely explanation for this localisation is that these structures are supplied with a very rich network of capillaries. Should this be the case, it affords an example of the distribution of an eruption being influenced by the local anatomical features of the skin. Whether, in addition to the skin manifestations, actual lesions are produced in the internal organs as a result of fungi lodging/

lodging in them has not yet been ascertained. Sulzberger(128) was unable to demonstrate any such lesions in his rabbit experiments. It is not known whether the hematogenous infection takes place early from the primary lesion, the fungi which reach the skin lying quiescent there and only producing lesions when a sufficient degree of allergic change has occurred in the skin cells, or whether blood spread occurs just before the appearance of the secondary eruption which is produced immediately the elements reach the skin. One point seems clear, that the development of allergy is essential for the occurrence of mycide lesions. The allergy modifies the morphology of the primary lesion in the first place, and it may be due to such a modification that the organisms are able to gain access to the blood stream. Secondly an allergic state of the skin is necessary before it responds with an intense inflammatory reaction to the presence of fungus elements brought into contact with it. The difficulty of obtaining positive blood cultures in a condition supposed to depend/

depend on a hematogenous mycotic infection is more easily understood in the light of the results obtained in animal experiments. Fungus elements have only been found in the secondary lesions at a very early stage in their development, and this can be explained on the ground that they are rapidly destroyed in situ by the inflammatory reaction which they precipitate in the allergic skin.

While the foregoing observations afford ample proof that mycides are caused by fungus elements brought to the skin by the blood stream, the rare occurrence of mycide lesions confined to the skin immediately surrounding the primary lesion raises the question as to whether, in such cases, the lesions have not been caused by an internal fungus infection, or by a spread from the primary via the lymphatics. By auto-inoculation experiments in human subjects White (93) has shown that secondary lesions can be produced, and persist, in which formed fungus elements rapidly disappear. For this reason he points out that the absence of fungus elements from secondary lesion of a trichophytide nature need not be interpreted as evidence that these have been produced by a hematogenous infection merely

on/

on account of their sterility. There is a considerable amount of evidence to show that mycides can be produced entirely by the action of the toxins emanating from the fungus in the primary lesion. As has been stated a generalised trichophytide eruption frequently follows the injection of trichophytine, and the localised reaction to trichophytine often has the characteristics of trichophytide lesions. Bloch (111) has proved conclusively that trichophytine alone can produce a generalised trichophytide eruption in the complete absence of any existing fungus lesion provided the skin has been rendered allergic as a result of previous mycotic infection. This method of production would explain the absence of fungi from the blood and lesions in such a large proportion of cases.

There are therefore three possible mechanisms by means of which the various trichophytides may be produced, namely (1) a hematogenous infection with fungus elements, (2) an intoxication with trichophytine, (3) a combination of both. It is possible that the ultimate cause of mycide lesions is in every case the action of fungus toxin/

toxin, even when fungus elements have been demonstrated in the blood stream and lesions. Leaving aside these finer details, the main factors necessary for the production of trichophytides are the opportunity for the fungi or toxins to enter the blood vessels, and the development of allergy. The former condition depends on the nature of the primary lesion which again is determined by the individual reactivity or allergic state of the skin. Allergic phenomena appear therefore to dominate the entire pathogenesis of trichophytides.

There is a close similarity between mycides and tuberculides both as regards their mode of production, clinical features, and associated biological phenomena. The mechanism underlying both is in all probability identical.

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SOME OBSERVATIONS ON A CONDITION OF CHRONIC ERYTHEMA OF THE LEGS.

BY

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ATTENTION has recently been drawn to the increasing prevalence in this country ([1], [2], [3]), in Denmark ([4]) and in Germany ([5]) of a vaso-motor disturbance of the cutaneous vessels of the lower extremities which has been observed exclusively in girls and young women. The condition is symmetrical, the region affected extending from the ankles to below the knees, and may either partially or completely encircle the limb. The skin in this area is thickened, and appears to be œdematous, though pitting on pressure is absent. It is of a diffuse bluish-red coloration, in severe cases being definitely cyanotic, and the pallor produced by pressure disappears immediately the pressure is removed. Hyperkeratosis is present at the pilo-sebaceous orifices, giving the appearance of "cutis anserina," and there is often an excessive growth of hair which is coarse in texture. Spontaneous ulceration does not occur, and there is no break in the skin surface unless as a result of trauma.

A prominent feature of the condition is the low surface temperature of the affected parts. The usual complaint in the slight and moderate cases is of this constant feeling of coldness, and of the unsightly appearance of the legs; pain is only present in the most severe types of the disorder. Itching is conspicuously absent.

Although the condition exhibits these well-defined features, it has not as yet received a definite and recognized title. It would seem, however, to belong to the erythemata, although, on account of the uncertainty which surrounds its ætiology, it cannot at present be accurately classed

in either the idiopathic or symptomatic group. Weber [1] suggests the name "chronic indurative erythema," and Meachen [6] has described it as "a persistent erythema of an erythromelalgic type."

There is no doubt that the incidence is almost wholly confined to a certain type of individual, the subjects of the condition being, as a rule, plump, flabby and inactive, and very similar in appearance to that class in which Bazin's disease is most frequently observed. In fact, Barber [7] makes the comment that in the absence of a similar type of sluggish circulation Bazin's disease rarely occurs; there does not appear, however, to be any suggestion of a tuberculous element in the production of the condition *per se*. Erythema pernio bears a close resemblance to it, and may actually occur on the areas of skin affected, but this condition is very intractable, and does not come and go as does erythema pernio, though certain ætiological factors appear to be common to both. There are also many features in common between this "erythema" and the less severe degrees of Raynaud's disease.

Since the colour of the skin depends on the amount of blood present in its capillaries and venules, and the surface temperature is regulated by the rate of blood-flow through the skin [8], it would appear that this condition is essentially one of vascular stasis, produced by simultaneous contraction of the arterioles and dilatation of the capillaries and venules. This may be brought about either by a loss of contractility in the vessel-walls, or by some derangement in the nervous mechanism whereby their calibre is regulated and their tonicity maintained.

A constitutional or hereditary defect in the vaso-motor mechanism may be an essential predisposing factor in the development of such a condition, several cases occurring in families having been recorded. From the situation of the lesion hypostatic influences might be expected to play some part in its ætiology.

There is much evidence in support of the theory, which has been advanced by several observers [1, 2, 3, 5, 9], that exposure to cold is the direct cause of the disorder, and the scanty protection to the legs afforded by modern female attire, by allowing ample opportunity for its operation, would also account for the sudden increase in the case-incidence.

Other observers [10, 11, 12] hold that an alternate cooling and heating of the skin surface, such as occurs when the chilled areas are roasted before a fire, is necessary for its production. Some experimental evidence may be quoted in support of these views. It has been shown [13, 14]

that in humans the exposure of the skin to low temperature produces a contraction of the arterioles, capillaries, and venules, with consequent pallor, and that prolongation of this treatment is followed by dilatation of the capillaries and venules, the arterioles remaining contracted. This results in a slowing of the blood-flow and a condition of local asphyxia, evidenced by the development of cyanosis. This subsequent capillary dilatation would appear to be due to the cold producing a partial paralysis of the contractile cells of the capillaries, the action being thus a direct one (15).

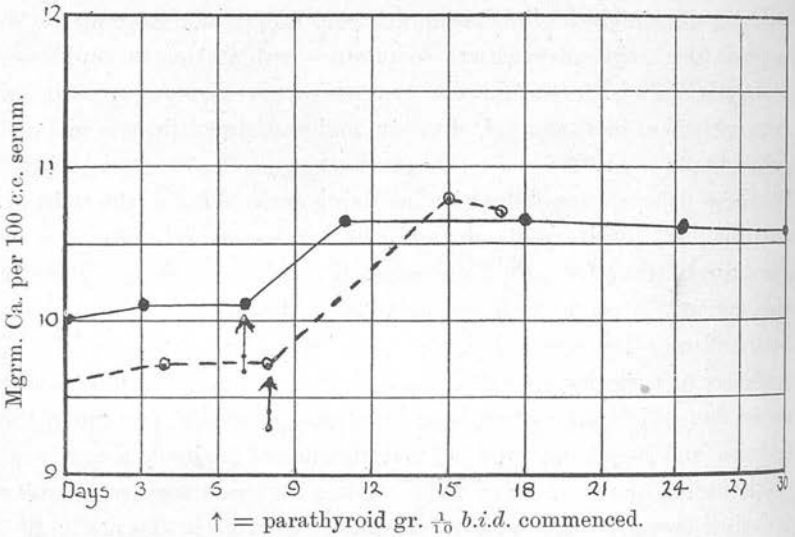
Studying the circulation in the rabbit's ear, Ricker and Regendanz (16) found that the action of heat was to produce a dilatation of capillaries and venules, which at the lowest temperatures employed passed off after some time had elapsed, but at higher temperatures remained permanently.

Endocrine defects, especially those involving dysfunction of the thyroid, parathyroid, and pituitary glands, and the ovaries, have been suggested as causative factors (17, 18, 19). In connection with the pituitary gland, it is of interest to note that Krogh (15) is of the opinion, from experimental evidence, that some substance which he suggests bears a strong resemblance to posterior pituitary gland, both in its properties and mode of action, but which has not yet been isolated, is normally present in the circulation, and is necessary for the maintenance of capillary tone.

Toxic bodies absorbed from the intestine or resulting from faulty metabolism have also been assigned an important *rôle* in the production of the vaso-dilatation.

The general use of calcium in the treatment of erythema pernio and the apparent relationship between this condition and that which is now being considered has led to the assumption that calcium deficiency may figure in the ætiology of the latter. This theory of hypocalcæmia receives support from the fact that calcium has a marked effect in contracting vessels when perfused through them (20), so that if it should be present in the serum in less than the normal concentration, a condition of vaso-dilatation might possibly be produced. The authors are in agreement with other workers (21) in having observed no deviation from the normal of the serum calcium content in erythema pernio, and not being satisfied that any significant alteration in this level could be produced by the amounts of calcium salts usually prescribed in its treatment, have also investigated the serum calcium in the present condition.

Using the method of Kramer and Tisdall ([22]) for the estimation of calcium in the blood-serum, this has been determined in 12 cases exhibiting the features already described. In none of these was the serum calcium found to be deficient in amount, the figures obtained ranging from 9·2 to 11·8 mgrm. per 100 c.c. serum. As we have estimated the normal value to be 9·2 mgrm. to 10·1 mgrm. per 100 c.c. serum ([23]), four of the cases examined showed a definitely high serum calcium. In 2 cases the diffusible calcium was estimated by a method of collodion filtration, and



↑ = parathyroid gr. $\frac{1}{10}$ *b.i.d.* commenced.

was found to amount to 73 per cent. and 80 per cent. of the respective total values—figures which are within the normal range.

In two cases, after a preliminary control period, parathyroid gland was given by the mouth and the effect on the serum calcium observed. Following the administration of this substance a well-marked rise in the total calcium content of the serum was noted, the extent of which is recorded graphically (see Chart). In spite of this rise no improvement in the patient's condition was evident.

From these observations we conclude that this condition does not supervene as a result of any deficiency in the serum calcium, also that an increase in the level of this is not followed by any amelioration of the symptoms, and therefore that therapeutic measures directed towards raising the serum calcium are uncalled for.

We desire to thank Dr. Frederick Gardiner and Dr. R. Cranston Low, who kindly put the necessary cases at our disposal. One of us (G.H.P.) holds a Grocers' Company's Research Scholarship, the other (C.P.S.) is in receipt of a grant from the Medical Research Council.

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HE CALCIUM CONTENT OF THE BLOOD-SERUM IN SKIN-DISEASES.

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CALCIUM salts have for some years been credited with very definite therapeutic properties in various skin conditions, notably in erythema pernio and in chronic leg ulcer. The grounds on which their efficacy has been judged have always been the clinical improvement which may have followed their use, but now, after a considerable trial, there appears to be some doubt as to their value. Furthermore, no definite explanation of the rationale of such a procedure is offered by its advocates, though it would seem to be inferred that a deficiency of calcium or a faulty calcium metabolism is the essential cause or predisposing factor in the conditions in which the treatment is recommended.

Very few observations have been made on calcium metabolism in skin-diseases, and those which are available have been confined to single estimations of the serum calcium in various conditions. Schamberg and Brown (1), Levin and Kahn (2) and Briggs (3) found the serum calcium to be within normal limits in psoriasis, acne, eczema and various other dermatoses. Schwartz and Levin (4), from an analysis of over 300 cases, conclude that there is no characteristic alteration in the serum calcium in diseases of the skin, though definitely low values were found by them in a considerable proportion of their cases of acne, eczema, actinic dermatitis, folliculitis barbæ, purpura, acne and furunculosis, and in the last-mentioned condition similar low values have been found by Thro and Ehn (5).

The serum calcium values obtained by the present authors in a variety

of skin conditions are presented in the following table. The method of analysis employed was that of Kramer and Tisdal, and the normal figure has been found to range from 9·2 mgrm. to 10·2 mgrm. per 100 c.c. serum. In the estimation of the diffusible fraction a method of collodion filtration, carried out as far as possible under constant conditions, was used.

TABLE 1A.

Disease.	Mgrm. calcium per 100 c.c. serum.	Percentage diffusible calcium.
Psoriasis	10·2	72·8
,,	9·8	65·5
,,	10·2	66·0
,,	10·0	60·0
Chronic erythema of legs	9·8	80·0
,, ,,	10·0	73·0
Erythema induratum	10·0	78·0
,, ,,	9·6	72·0
Ulcus cruris	9·8	65·0
,, ,,	10·4	70·0
Acne pustulosa	10·2	62·0
Seborrhœic dermatitis	10·1	64·0
,, ,,	10·1	78·0

The figures recorded are in agreement with those of previous observers, and do not suggest that in the commoner skin-diseases there is, on the whole, any great departure from the normal of the serum calcium content, any variations noted being as common in one direction as in the other. Variations in the diffusible portion lie within normal limits in the cases in which this fraction has been determined. Vines ([6]), on the other hand, reports low figures for the diffusible serum calcium in chronic leg ulcer, the total calcium being normal. The difficulty in estimating this fraction naturally renders any results open to criticism, but a standardized method of collodion filtration, such as was used by us, yields results which are comparable and in our experience of greater accuracy than does the method of precipitation used by the former worker.

It will be noted that in one case of Raynaud's disease and of papulo-necrotic tuberculide, and in two cases each of purpura and lupus erythematosus, the serum calcium was distinctly low, being less than 9 mgrm. per 100 c.c. serum. Since definite malnutrition was present in these cases, either coincident with or resulting from the disease, we are of opinion that

TABLE 1B.

Disease.	Mgrm. calcium per 100 c.c. serum.
Lupus vulgaris	10·3
,,	10·0
,,	9·5
,,	9·4
,,	9·2
,,	9·2
Dermatitis	9·8
,,	10·1
,,	9·5
,,	10·4
,,	9·6
,,	9·6
,,	9·8
,,	10·6
,,	10·4
,,	12·0
Varicose dermatitis	13·0
,,	12·7
Erythema pernio	10·0
,,	9·2
,,	9·8
,,	9·3
,,	9·2
Raynaud's disease	8·5
,,	11·6
Lichen planus	10·3
,,	10·4
,,	10·42
Purpura	6·9
,,	8·8
,,	9·8
Chronic erythema of legs	10·0
Ditto	10·4
,,	10·4
,,	9·8
,,	10·9
,,	9·2
,,	9·89

Disease.	Mgrm. calcium per 100 c.c. serum.
Chronic erythema of legs	9·5
Ditto	9·4
,,	10·2
,,	9·4
,,	9·8
Lupus erythematosus	10·2
,,	10·5
,,	10·9
,,	9·58
,,	8·0
,,	8·6
,,	9·2
Ulcus cruris	9·58
,,	10·42
,,	9·98
,,	10·4
,,	9·89
,,	9·8
,,	9·6
,,	10·2
,,	11·5
,,	12·0
Papulo-necrotic tuberculide	8·5
Psoriasis	10·7
,,	10·7
,,	10·4
,,	10·2
,,	10·0
,,	10·0
,,	11·2
Erythema induratum	10·6
,,	9·9
,,	9·8
,,	9·5
,,	9·2
Acne vulgaris	9·9
,,	9·8

the diminished serum calcium was rather one manifestation of a general condition than a factor in the ætiology of the cutaneous disorder, and we would suggest a similar explanation for the low figures obtained by Schwartz and Levin and by Thro and Ehn, in whose cases showing low calcium values a malnutrition syndrome may well have existed. A similar explanation is given by Ashford and Hernandez ([7]) for the decreased serum calcium found by them in a proportion of cases of sprue and amongst the insane, and they express the view that "low calcium values in the blood-serum are to us indicative of severe malnutrition rather than of specific disease."

From the fact that a diminution or increase in the serum calcium concentration has not been found to be a constant feature of any of the dermatological conditions which have so far been examined, it would appear that such variations as are found do not result from any significant derangement of calcium metabolism, since in the disorders known to be due to such a metabolic error well-marked alterations in the serum calcium are always present.

To test this hypothesis it was decided to induce alterations in the serum calcium in various dermatoses by the administration of parathyroid extract which Collip ([8]) and others ([9]) ([10]) have shown to be capable of raising the serum calcium in animals, and which the present authors ([11]) have shown to have a similar effect on the human subject. This action is stated to be accompanied by an increased urinary excretion of calcium ([12]), but whether the changes are due to an increased absorption of calcium or to the tissue stores being drained and ultimately depleted is not yet known. Either mechanism would, however, bring about a definite alteration of calcium metabolism, or an exaggeration of the normal processes.

This procedure was carried out in several cases of Bazin's disease, chronic erythema of the legs, chronic leg ulcer, psoriasis, acne pustulosa and lupus erythematosus. A very definite rise in the total serum calcium was obtained in all the cases mentioned accompanied by a coincident rise in the diffusible portion, but neither was clinical benefit observed to follow this alteration, nor was the severity of the condition increased.

It may therefore be inferred that in the diseases mentioned there is no ætiological relationship between deranged calcium metabolism and the onset and subsequent course of the condition.

The cases used in this investigation were kindly placed at our disposa

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THE SULPHYDRYL-CONTAINING CONSTITUENT OF THE EPIDERMIS AND ITS RELATIONSHIP TO MELANOGENESIS AND KERATINIZATION.

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INTRODUCTION.

FOLLOWING the work of Goffa on the occurrence in certain vegetable cells of a substance giving a red-violet coloration with alkaline sodium nitroprusside, Buffa found that a similar substance was widely distributed in mammalian tissues. Amongst those giving the "rose-carmine" coloration with sodium nitroprusside after soaking in 10% sodium hydroxide was the epidermis, and though the coloration was too fleeting for a detailed study of the exact location, he thought it was most intense in the rete mucosum. The dermis remained unstained. The hair-bulbs were deeply coloured, the sheaths less so, and the hair-shafts, like the nails, gave a violet colour. Buffa thought the "rose-carmine" colour was due to the presence of cystein, and indicated great metabolic or proliferative activity of the protoplasm, while the violet colour of the nails and hair-shafts indicated the presence of "alkaline sulphur compounds," possibly of cystine.

By 1921 it had become generally recognized that part, at least, of the oxidative activity of living cells was dependent on the presence of soluble substances containing a sulphydryl group and consequently giving a

* Working for the Medical Research Council.

reddish-violet colour with sodium nitroprusside in weakly alkaline solution. In that year Hopkins described the isolation of "glutathione," which he considered to be the substance responsible for this coloration and extractable from tissues by water. Recently (Hopkins, 1929) it has been shown that glutathione is a tripeptide of cystein, glycine and glutaminic acid, and that the earlier preparation believed, on the basis of work by Hopkins (*loc. cit.*), Quastel Stewart and Tunnicliffe (1923), and Stewart and Tunnicliffe (1925), to be a dipeptide of cystein and glutaminic acid, was impure. The pure tripeptide is unable to restore oxidative activity to washed tissue, but does so when mixed with a small amount of cystein (Meldrum—private communication). Hence it appears that the glutathione system consists of the tripeptides, together with another substance, possibly free cystein.

Hopkins's work made it desirable to reinvestigate the sulphydryl-containing substance of the skin, and in 1924 Walker confirmed Buffa's observation that it was limited to the living cellular parts of the epidermis, and showed by various chemical modifications of the staining method that the substance giving the colour with sodium nitroprusside really contained a sulphydryl group, and was not one of the various other compounds—*e. g.* aceto-acetic acid—which react similarly. Kaye (1924) arrived at similar conclusions, but considered the substance to be glutathione. This last statement Walker (1925) considered to be incorrect, for he was unable to extract the sulphydryl-containing substance with water, whereas glutathione is easily soluble in water and is readily extracted, *e. g.* from minced muscle or yeast. Kaye herself, indeed, had failed to extract the substance by the ordinary method, but stated that it was removed by running water. Giroud and Bulliard (1929) accepted Kaye's conclusion as to the nature of the substance, rejecting Walker's evidence to the contrary, and not, themselves, advancing further evidence of any kind.

The distribution of the nitroprusside stain in skin and hair—where she found the colour more intense in the bulbs of pigmented than of grey or white hairs—led Kaye to the view that the reaction is intense where there is active metabolism, but that the substance responsible may be present only in very low concentration where metabolism is excessive or pathological.

Giroud and Bulliard, who used the same technique as the previous workers, have made a more detailed study of the distribution of the

reaction. They found that the reaction is given by the epidermis, hair-follicle and root-sheaths, hair-bulb, sebaceous and sweat-glands, arrectores pilorum, and the walls of the arteries. The colour obtained was most intense in those areas immediately preceding the appearance of keratin, in which the reaction was absent. Thus an intensification occurred in the epidermis in the region of the stratum granulosum, and the reaction ceased abruptly when at its maximum just below the stratum corneum. A similar intense reaction was observed in the hair-bulb. This rapidly faded towards the shaft, which gave no reaction. In the nail the reaction was strongest just underneath the nail-plate in the matrix region. These observations are illustrated diagrammatically, presumably because the rapid disappearance of the colour reaction rendered photography impossible. In addition to regarding the SH group as glutathione or a closely related substance, these authors put forward the suggestion that it is intimately associated with the process of keratinization, and that it may even be the precursor or mother substance of keratin. They pointed out that this supposed transformation occurs abruptly when keratohyalin takes part in keratin formation, and gradually in the absence of kerato-hyalin. This is illustrated by the different distribution of the reaction in the epidermis and in the hair. They found no relationship between the degree of pigmentation of the skin and the intensity of the nitroprusside reaction, nor was the reaction altered in skin showing parakeratosis.

The present series of experiments was undertaken with a view to ascertaining more clearly the distribution of the reacting substance in the skin, and its relationship, if any, to the processes of melanogenesis and keratinization.

Technique of Nitroprusside Staining.

Previous workers have all been handicapped by the rapid fading of the colour given by skin sections with sodium nitroprusside. Normally the colour fades in about two minutes, even when ammonia is used as the alkali; with sodium or potassium hydroxide it disappears even more rapidly. Di Mattei and Dulzetto (1928), in an attempt to prolong the time available for examination, soaked the sections in trichloracetic acid, then stained in the ordinary way, maintaining a low temperature throughout, and examined at 5° C. In testing solutions for the presence of sulphydryl groups, it has been found that the colour was adsorbed and rendered less labile by zinc hydroxide, and this observation we have

applied to the study of skin sections. Frozen sections, about 40μ thick, are floated on to microscope slides with as little soaking in water as possible. (In thin sections the sulphydryl group is rapidly oxidized by the oxygen dissolved in the water.) They are then treated successively with 10% zinc chloride, 5% sodium nitroprusside in 15% ammonium sulphate, and 2% ammonia. The reagents are added from a dropping-pipette, and each is added about 30 seconds after the preceding one. It is not necessary, though, owing to precipitation of zinc hydroxide, it is perhaps desirable, to pour off each reagent—without washing—before the next is added. Finally the section is floated to a clean slide and mounted in *Farrant's solution*. With this technique the colour is not absolutely permanent, but does not fade appreciably in four hours, and remains sufficiently intense to allow of examination for a day or two. A few preparations have shown the colour almost unimpaired at the end of a week.

Distribution of the Sulphydryl-containing Substance.

(A) *In Normal Skin and Hair.*

This technique was applied to fresh portions of human skin obtained from different parts of the body, including the sole of the foot, the abdomen, the axilla, the scalp and the back. No marked difference in the intensity of the reaction was noted between these regions, although the skin of the sole of the foot probably reacted most strongly and the staining could be studied most conveniently in that situation (Fig. 1). To the naked eye what appeared to be the deeper part of the epidermis stood out as a bright pink to violet band, and in the hairy regions the hair-follicles could be seen as pink streaks. It was found microscopically that the normal stratum corneum and the dermis did not give the reaction, and that it was present in the rete mucosum, the hair-follicles, bulbs and sheaths, the arrectores pilorum muscles, the arterial walls and the sweat-glands. In the epidermis the whole of the rete mucosum was coloured and the colour became progressively deeper from the basal layer towards the stratum granulosum, being most intense in and just beneath this layer. The reaction stopped abruptly at the stratum lucidum, which, along with the stratum corneum, was unstained. These gradations in the intensity of the reaction were most evident in sections made from the sole of the foot. Here the cells of the granular layer were of a deep pink colour.

Fig. 2.—

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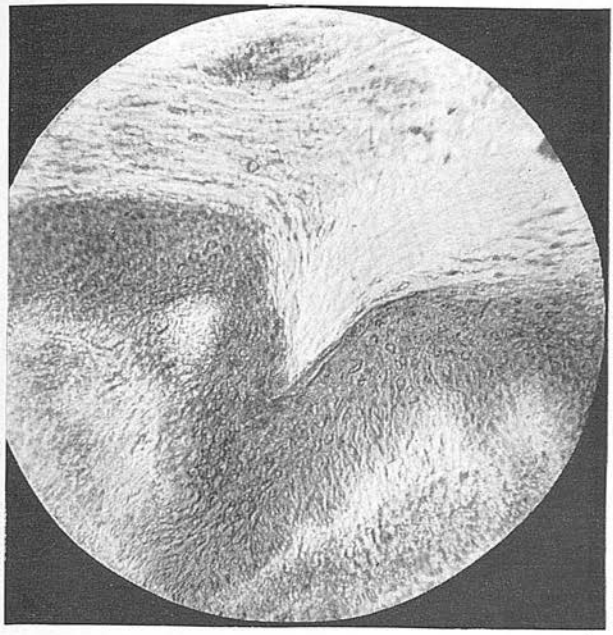

FIG. 1.—Nitroprusside reaction in epidermis from the sole of the foot.

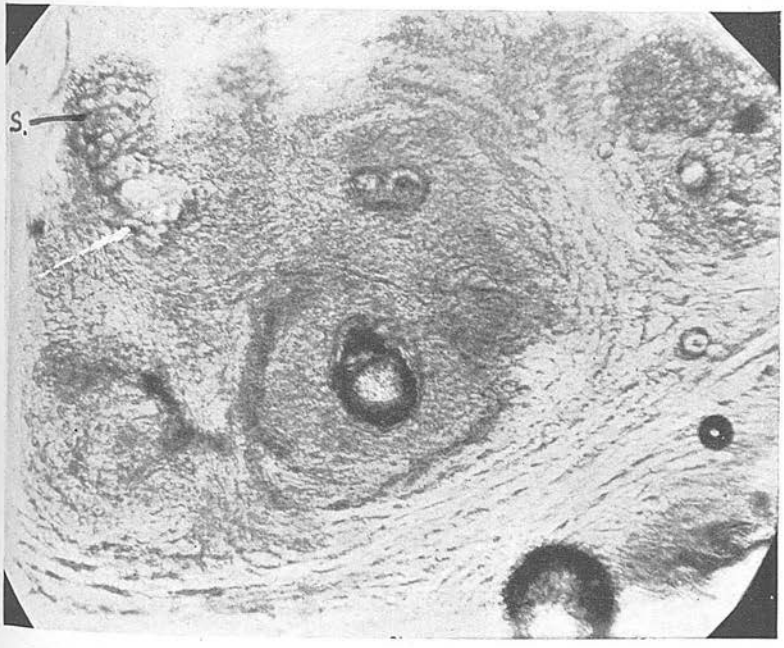

FIG. 2.—Nitroprusside reaction in a hair-follicle and sebaceous gland (**S**).

TO ILLUSTRATE ARTICLE BY DR. PERCIVAL AND DR. STEWART ON THE SULPHYDRYL-CONTAINING CONSTITUENT OF THE EPIDERMIS.

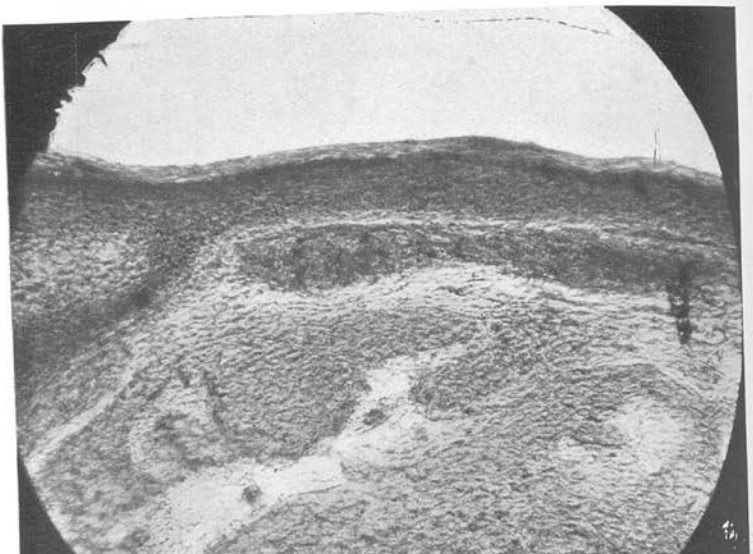

FIG. 3.—Nitroprusside reaction in a basal-cell carcinoma.

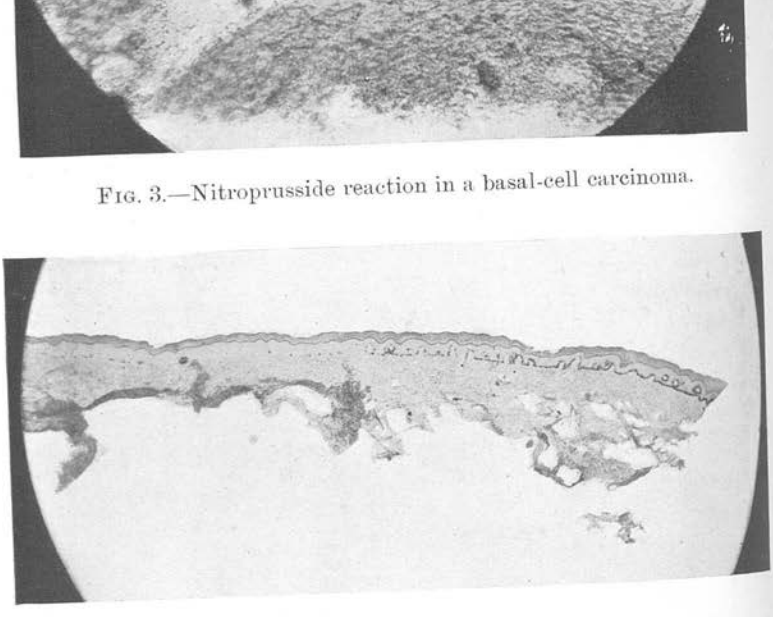

FIG. 4.—Dopa reaction in vitiligo.

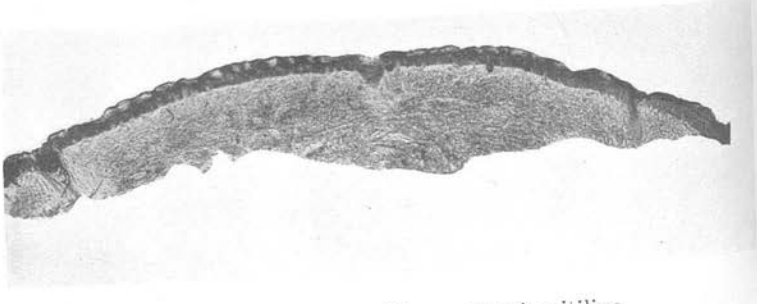

FIG. 5.—Nitroprusside reaction in vitiligo.

TO ILLUSTRATE ARTICLE BY DR. PERCIVAL AND DR. STEWART ON THE SULPHYDRYL-CONTAINING CONSTITUENT OF THE EPIDERMIS.

and the subjacent rete cells were a paler tint of brownish-red. In sections from other areas the rete was stained a bright pink, which increased in depth towards the surface. The entire cell cytoplasm was uniformly coloured. Transverse and vertical sections of the scalp showed that the hair-follicles and sheaths gave an equally intense reaction. The bulbs were more deeply coloured, and the shafts were unstained. The cells lining the sebaceous glands were also stained, but their secretion did not react (Fig. 2). The hair-bulb and sheath were more easily studied on epilated hairs in which the outer and inner root sheaths stained uniformly, while the cells of the bulb gave a more intense reaction. The deep pink colour of the bulb faded rapidly towards the shaft, which was coloured to a slight extent in its lower portion only. The bulbs of pigmented hairs seemed to give a more intense reaction than those of white hairs, but the difference was slight, and probably due to the added depth lent by the pigment itself.

Sections of the buccal mucosa were treated with alkaline nitroprusside solution, and it was found that the epidermis in the whole of its width gave a deep pink reaction. Throughout the entire epidermis the colour was perfectly uniform, and there was no evidence of either concentration or fading of the reaction in the more superficial cells. The presence of an undiminished reaction in the most superficial layers of the epidermis of the mucosa points to the negative reaction found in the stratum corneum of the epidermis being due to an active oxidation of the SH substance taking place in the epidermal cells, and not merely to its oxidation by atmospheric oxygen.

(B) *The Sulphydryl containing Substance in Abnormal Skin.*

As it was difficult to determine to what extent the reaction was present in the single-celled basal layer in ordinary sections, tissue from a basal cell carcinoma was examined (Fig. 3). It was found that the basal cells forming the dermic infiltrate stained less deeply than the cells of the rete mucosum as a whole, and that the deeper the situation of the infiltrating cell, the less intense was the coloration obtained.

In view of the absence of the reaction in the normal stratum corneum, it seemed desirable to examine specimens of skin exhibiting abnormal forms of keratinization. Psoriasis scales and sections of psoriasis lesions were treated with alkaline nitroprusside solution, and it was found in

both cases that a violet-red colour developed in patches throughout the scales and parakeratotic stratum corneum.

Dyskeratotic cells of molluscum contagiosum were examined in material expressed from molluscum lesions. This material included the deeper cellular structure of the tumours, and also numerous molluscum cells. In all the preparations the whole of the tissue gave a moderately strong nitroprusside reaction with the exception of several isolated molluscum cells where the reaction had in all probability been present, but was either too faint or too fleeting in character to be apparent microscopically. Similar tissue treated with a 30% solution of potassium hydroxide gave a red reaction on the addition of the nitroprusside solution. Although little is known of the exact significance of these dyskeratotic molluscum cells, their main morphological character is their failure to undergo the usual changes in the stratum granulosum, and to assume the form of normal keratinized cells. It was for this reason that molluscum tissue was chosen for examination as an example of abnormal keratinization.

The Nature of the Sulphydryl-containing Substance in the Epidermis.

Glutathione, which Kaye (1929) has supposed to be responsible for the nitroprusside reaction in the skin, is very readily soluble in water. Indeed it was this property which first drew attention to the importance for cell respiration of a sulphydryl containing substance, and glutathione was isolated from an aqueous extract of yeast. It is equally soluble in dilute acid, and the pure tripeptide is prepared from a dilute acetic acid extract of, *e. g.*, yeast. It is readily removed from minced muscle by extraction with water, and can be found in the extract. Kaye failed to extract the sulphydryl-containing substance of the skin by shaking with water, but stated that it was extracted by running water, since after that treatment the skin no longer stained with nitroprusside. Obviously the failure to stain might have been due merely to oxidation of the sulphydryl group, and it was impossible to prove the extraction of the SH substance by finding it in the extract. Walker (1925), who also failed to extract the SH substance with water, drew the more logical conclusion—that the substance responsible for the reaction was not glutathione.

We have repeatedly attempted, without the slightest success, to extract the sulphydryl-containing substance of skin with water, and with dilute

acid. In one experiment, a large number of skin-sections were placed in about 10 c.c. of water, and the mixture was boiled ; the extract gave no nitroprusside reaction, even after treatment with a reducing agent, though with a similar amount of muscle the glutathione could be detected in the extract. In another, after three days' extraction with boiled water, the mixture being frequently shaken, the extract gave no colour with nitroprusside, while the skin still stained strongly. When the skin was extracted with water through which air was bubbled, the power to stain with nitroprusside was slowly lost, but again the aqueous extract contained no substance possessing a sulphydryl or disulphide group.

These facts, we consider, show conclusively that the sulphydryl group of skin is not part of a glutathione molecule, but is similar to what Hopkins terms the " fixed SH " of muscle, *i. e.* attached to protein.

Though this conclusion, if correct, denies to the SH-containing substance of the epidermis any special catalytic action in the production either of keratin or melanin, since the " fixed SH " of muscle appears to have no respiratory function save in presence of glutathione, it remains possible that it may be a precursor of keratin, though probably not of melanin, which contains no sulphur. Keratin is peculiarly rich in sulphur, which, as Rimington (1929) has shown, is, at any rate in the case of wool, entirely present as cystine. We have found accordingly that the stratum corneum of the skin, though it ordinarily gives no coloration with nitroprusside, does so after reduction—whereby cystin is reduced to cystein. In this connection, however, a curious phenomenon was observed. When the action of sodium on water was used for the reduction, subsequent treatment with nitroprusside gave the usual reddish-violet colour in the stratum corneum, but a blue-violet with hair-shaft and with nail. Direct treatment with nitroprusside and ammonia gave staining in none of these situations. Later it was found that by soaking the sections in 20% NaOH (or KOH) the same bluish colour was obtained on staining hair or nail, while stratum corneum gave a weak reddish violet, which, however, tended to become blue with prolonged soaking in alkali. The keratin of the stratum corneum, then, was so altered by alkali that, possibly *inter alia*, the disulphide groups were reduced to sulphydryl. In hair-shaft and nail, however, a different change was produced, and since hydrogen sulphide itself gives a blue colour with sodium nitroprusside, we suspected that this substance had actually been produced. This suspicion we confirmed by soaking white hair-shaft and sections of nail in a solution

of lead acetate in 20% KOH. There quickly appeared a brown stain—lead sulphide—whereas sections of stratum corneum similarly treated showed no appreciable darkening.

Relation of the Sulphydryl of the Epidermis to Melanogenesis.

The conclusion of previous authors regarding the nature of the substance responsible for the nitroprusside reaction in the skin and the interpretation of the physiological significance of this reaction raised the question of its relationship to melanogenesis. Though, *à priori*, no such relationship is to be expected in view both of Raper's work on melanogenesis in plants and of our findings as to the nature of the sulphydryl-containing substance in skin, the possibility was studied microscopically in various tissues which had been subjected to the nitroprusside, dopa and silver reactions, and also stained by hæmatoxylin and eosin. The tissues included skin from a case of vitiligo, skin which had been exposed to ultra-violet irradiations of varying intensity, and that from a pigmented nævus. The silver reaction was carried out by the usual method to demonstrate the presence of melanin.

The Technique of the Dopa Reaction.

The method of dopa staining we have used is exactly that of Bloch (1927), but as it has never been published in this country, it may perhaps be detailed here. The essential part of the method is the exact adjustment of the pH, which must not be so acid as to prevent the oxidation of the dopa in the pigment-forming cells, while it must not be so alkaline as to allow the oxidation to proceed in the solution itself by the aid of atmospheric oxygen alone ; the margin of safety is narrow.

For buffering, a mixture of potassium dihydrogen phosphate and disodium hydrogen phosphate is used, the correct proportion being determined by the method of trial and error. For this purpose a M/15 solution of the former (A) and a M/15 solution of the latter (B) are prepared, using water prepared in the following way. Using Jena glass apparatus throughout, the water is distilled ; the distillate is again distilled with potassium permanganate (2 grm. per litre) ; the distillate from this is acidified with 85% orthophosphoric acid (4 grm. per litre), and distilled; this distillate is again distilled after addition of anhydrous sodium carbonate (12 grm. per litre) ; and finally the distillate is once more distilled

alone. The water is stored in Jena glass flasks under sterile conditions. It is boiled immediately before use.

A series of mixed solutions is then prepared, using one volume of "A" to 3, 4, 5, etc., volumes of "B." (We found the optimum mixture to be 1 volume of A to 5 volumes of B.) A solution of dopa (obtained from Hoffmann la Roche) containing 1 mgrm. per c.c. is prepared, using the specially distilled water, freshly boiled, and adding the dopa while the water is still warm. To a dish containing 2 c.c. of dopa solution is added two drops (0·1 c.c.) of buffer solution, and after mixing, a few sections of skin. (The skin, after hardening in neutral formalin for 1 to 2 hours, is frozen and cut in sections 20μ thick.) The dish is allowed to stand at room temperature for 24 hours or at 37° C. for 4 hours, and representative sections are then mounted in Canada balsam and examined. That mixture of phosphates is taken as standard, and used for future work, which has allowed the full staining of the pigment-forming cells with the minimum general darkening of the section and of the surrounding solution. It will be recalled that not only does the dopa reaction indicate the pigment-forming ability of a cell, but that it demonstrates the presence of dendritic cells in the basal layer of the epidermis, hair-follicles and matrix. These dendritic cells are dopa-positive. They are probably basal cells which have acquired dendritic processes as a result of some pigment-forming stimulus, and there is a good deal of evidence to suggest that the presence of dendritic forms coincides with a very high degree of active pigment-formation.

Comparison of Highly Pigmented and Poorly Pigmented Skin.

Deeply pigmented skin from the back which had been irradiated with a carbon arc light was compared with poorly pigmented skin from the axilla of the same subject. The specimens were subjected only to the dopa and nitroprusside reactions. In the poorly pigmented skin there were approximately 9 dopa-positive cells per field in the basal layer, and these reacting cells were morphologically basal cells. In the deeply pigmented skin there was an average of 19 dopa-positive cells per field, most of which were large basal cells with short dendritic processes extending upwards into the rete. A few large well-formed dendritic cells were also present. No difference in the intensity of the nitroprusside reaction was observed betwixt the highly pigmented and poorly pigmented samples of epidermis.

Results in a Case of Vitiligo.

A portion of skin, including a pigmented and depigmented area, was obtained from a vitiligo patch on the back of the hand. Unstained sections showed the presence of abundant pigment-granules in the cells of the basal layer at one end of the section and slight pigmentation at the other end. The central portion contained no pigment. The silver nitrate preparation accentuated this variation in pigment distribution. The dopa reaction was strongly positive in the basal layer at one end of the section and stopped abruptly towards the centre (Fig. 4). The other extremity contained a few dopa-positive cells, while in the central area the reaction was negative. The rete Malpighii throughout the entire section gave a bright pink nitroprusside reaction (Fig. 5). The hæmatoxylin and eosin-stained sections showed a normal epidermis with a well-marked stratum corneum. The dermis showed some dilatation of the papillary vessels and of the subpapillary plexus, accompanied by a scanty round-cell infiltration and an increase in the connective-tissue cells.

There was therefore complete absence of pigment and pigment-forming capacity in the basal cells in certain regions, as evidenced by the silver and dopa reactions respectively. Contrasted with these variations in pigment content and activity which occurred side by side, the SH compound demonstrated by the nitroprusside reaction was uniformly present throughout the whole of the basal layer and rete Malpighii.

Pigmented Nævus.

The results obtained in a pigmented nævus with these reactions were similar to those in vitiligo. Hæmatoxylin and eosin sections showed a massive dermic invasion of clumps and columns of nævus-cells. In the stroma deeply pigmented cells were seen singly and in small clusters in unstained and silver-stained sections. The cells of the large masses contained very little pigment. The dopa reaction was positive in many of the cells at the periphery of the infiltrating columns, but few of the more centrally placed cells gave the reaction. The deeply pigmented cells in the stroma gave a negative dopa reaction. The nitroprusside reaction was present and of uniform intensity throughout the whole of the nævus, excepting the heavily pigmented dopa-negative cells in the interstices of the infiltrating clumps of nævus cells.

These observations afford no evidence of a functional relationship between pigment-formation and the presence of an SH grouping in the

cell protoplasm. Cells which are capable of immediate pigment-formation or which are in the process of producing pigment, as evidenced by a positive dopa reaction, do not appear to be richer in SH grouping than those in which pigment function is absent or in abeyance.

Reaction to Light.

The effect of ultra-violet irradiation on the SH grouping in the epidermis, on the dopa reaction and on epidermal growth and keratinization was next studied in the human skin.

A circumscribed oblong area of skin on the back was given an erythema dose of ultra-violet rays, the surrounding skin being protected from their action. Two days later portions of skin from the screened and irradiated areas were removed and examined. In the non-irradiated skin an average of 8 cells per field were dopa-positive, and only a few of these showed rudimentary processes. In the irradiated portion an average of 14 cells per field were dopa-positive. These dopa-positive cells were larger than those in the non-irradiated cells, and about 10% possessed well-marked dendrites. A similar increase in the ratio of dendritic to non-dendritic cells has been observed by Peck (1930) following irradiation of the skin with thorium X. The nitroprusside reaction was of equal intensity in the irradiated and non-irradiated skin.

On the eighth day after irradiation portions were again excised from the irradiated and non-irradiated areas and subsequently examined. In addition to applying the dopa and nitroprusside tests, the width of the rete Malpighii and stratum corneum was measured in hæmatoxylin and eosin preparations to ascertain if any increase in the rapidity of cell-division in the epidermis as evidenced by an increase in the thickness of these layers had been caused by the irradiation. The dopa reaction was present in sections from both areas. In the non-irradiated area the number of dopa-positive cells averaged 9 per field, and all of these were morphologically basal cells. In the irradiated portion there were, on an average, 13 dopa-positive cells per field, and while they all possessed rudimentary dendritic processes, some presented a fully developed dendritic structure. No obvious difference between the amount of formed pigment present in the two areas was observed in silver nitrate preparations. In the non-irradiated portion the stratum corneum had an average width of 0·009 mm., and the supra-papillary portions of the rete Malpighii 0·033 mm. The stratum corneum in the irradiated skin measured 0·027

mm. and the rete Malpighii 0·051 mm. Thus, coincidentally with an increase in the number of cells capable of immediate pigment-formation, both cell division and keratinization had been markedly augmented. The nitroprusside reaction was of equal intensity in the irradiated and non-irradiated portions.

This absence of any relationship between the nitroprusside reaction and the phenomenon of increased cell division in the epidermis following ultra-violet irradiation was verified in two subsequent experiments. In these the width of the epidermis, the nitroprusside reaction and the dermic changes were studied and compared in normal and irradiated skin. In one experiment the normal rete Malpighii was 0·033 mm. in width and the stratum corneum 0·009 mm. In the adjacent portion of epidermis, which had received an erythema dose of ultra-violet rays five days previously, the rete Malpighii measured 0·0738 mm. and the stratum corneum 0·0209 mm. In the irradiated portion mitoses were numerous in the basal layer and the stratum granulosum stained more deeply, although there was no noticeable cellular increase in this layer. Nucleated cells were present here and there in the stratum corneum. The papillæ were œdematous and there was a perivascular infiltration, which consisted of small and large lymphocytes, endothelial and connective-tissue cells, situated round the dilated capillaries.

The nitroprusside reaction was of equal intensity in the irradiated and non-irradiated portions.

The second experiment was carried out on the same lines and gave similar results. The normal rete Malpighii and stratum corneum measured 0·0276 mm. and 0·0075 mm. respectively. The irradiated rete measured 0·039 mm., and the stratum corneum 0·0123 mm. The same histological changes in the epidermis and dermis were seen as had been noticed in the previous experiment. There was no difference in the intensity of the nitroprusside reaction given by the two areas. In this experiment special staining of the elastic and connective tissue of the dermis did not reveal any difference in amount or staining reaction between the irradiated and non-irradiated portions.

Discussion.

The nitroprusside reaction is given by many cellular structures, and is not peculiar to the cellular elements of the skin. When present it appears to be a property only of the nucleated and presumably living cell. This

is demonstrated in the epidermis, where the reaction is absent from the normal stratum corneum, the hair-shafts and the disintegrated cells which form the secretion of the sebaceous glands.

The results obtained in the present work do not support the idea of a relationship between the sulphydryl group and the pigmentary function of the skin. There is no increase in the concentration of the sulphydryl-containing substance when the pigment-forming activity of the skin is increased by exposure to ultra-violet light; nor is there any decrease in skin whose pigment-forming activity is markedly subnormal, *e. g.* in vitiligo. Moreover, since melanin has been shown to contain no sulphur, it is unlikely that the sulphydryl-containing substance is a precursor of melanin, nor, probably, does it play a part in the oxidation leading to the production of melanin, since it appears to correspond to what Hopkins has termed the "fixed SH" of muscle, which has no direct oxidative activity, and serves merely to maintain the concentration of reduced glutathione—and glutathione appears to be absent from skin.

Miescher (1929) has recently drawn attention to an important defensive mechanism of the skin against light-rays. Protection of the rete Malpighii against the effects of over-exposure to these rays is brought about, not by an increase in the pigment content of the basal layer, but by an increase in the thickness of the stratum corneum. Our own work shows such a thickening of the stratum corneum to follow exposure of the skin to ultra-violet light, and any participation of the sulphydryl group in the light-protecting mechanism must be connected with this hyperkeratinization, *i. e.* with its action as precursor or catalytic agent, in the formation of keratin. Yet we cannot find that the sulphydryl group plays any part in this type of reaction to light, during the course of which, it may be remarked, the rate of cell division and metabolism must be greatly increased. There is no increased concentration of the sulphydryl group in skin which has been exposed to ultra-violet light and which shows increased keratinization in progress, and there is no difference in the concentration of the sulphydryl group—as shown by the intensity of the colour given with sodium nitroprusside—corresponding to the thickness of the horny layer. The mucous membrane of the bucal cavity gives as intense a colour as skin from the abdomen, the axilla or even the sole of the foot. Hence the concentration of the sulphydryl group does not seem to be related to the prevailing metabolic activity of the epidermal cells—a conclusion which is supported by the fact that a basal cell

carcinoma and a benign melanoma gave no very intense coloration with sodium nitroprusside.

It is obvious that the sulphydryl group has no catalytic function in the formation of keratin. Apart from the constancy of its concentration with varying rates of keratin formation, this conclusion is supported by the considerations already detailed in connection with melanin formation. Further, the sulphydryl group is found in normal amount in the rete mucosum of skin presenting abnormal keratinization.

Nevertheless, keratin is a protein rich in sulphur, and it may well be that the sulphydryl-containing protein of the epidermis is a precursor of keratin. An indication of this is found in the fact that in psoriasis and molluscum contagiosum the abnormal surface cells still give the nitroprusside reaction. Moreover, the normal stratum corneum, on reduction, shows the presence of sulphydryl groups. The negative reaction of the normal stratum corneum is not due merely to oxidation by atmospheric oxygen, since in psoriasis normal surface cells show no reaction, while abnormal cells, with equal exposure to air, give a positive reaction. Moreover, the buccal mucosa gives a positive nitroprusside reaction in its most superficial layers.

There appears to be some essential change from the pro-keratin of the rete mucosum or hair-bulb to the fully formed keratin of the stratum corneum or hair-shaft—a change involving, *inter alia*, the oxidation of the sulphydryl group to a disulphide group. In the epidermis this chemical change is evidently closely associated as regards time and place of occurrence with the morphological change undergone by the cells in the stratum granulosum; in the buccal mucosa, with no stratum granulosum, the chemical change fails to take place. It is significant that the keratin of the hair-shaft is not identical with that of the stratum corneum. Both contain the disulphide group, and both on reduction show sulphydryl groups, but the sulphur of the hair-shaft is relatively labile and is removed by potassium hydroxide. Whether the different keratins, the one type in the stratum corneum of the epidermis, the other in the hair-shaft and the nail, are due to the existence of different precursors or are produced from the same pre-keratin by different processes it is impossible to decide.

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MELANOGENESIS: A REVIEW.

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UNTIL recently several divergent opinions have been held regarding the nature and origin of melanin pigment in man and the lower animals. Although the subject still presents many unsolved problems, considerable unanimity of opinion has been reached regarding the fundamental processes which it involves, and controversy now centres round the finer details of a generally accepted view. A notable advance has thus been made towards the final solution of the problem. As several résumés of the older and now discarded theories of the process of melanin formation (melanogenesis) are to be found in the literature (Bloch, 1927; Lindberg, 1923; de Juste, 1929), it is unnecessary here to refer to them in detail. This paper will therefore be limited to a review and a discussion of the present-day conception of melanogenesis, and of the recent advances which have been made in connection with it.

Situation of Naturally Occurring Melanin. — Melanin pigment is found in the skin and its appendages. In this situation the melanin content presents great individual, racial and species variation, and it is also capable of undergoing wide alterations in a given subject, as a result of physiological or pathological stimuli. Apart from the skin, melanin occurs in the eye, where it is situated in the choroid, retina, ciliary body and iris, and in the central nervous system in the substantia nigra, and scattered throughout the meninges.

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In white human skin, melanin appears as light yellow to brown-coloured granules in the cells of the basal layer of the epidermis, and also in a few cells of the adjacent rete malpighii (Fig. 1). Treatment with silver nitrate solution renders these granules darker in colour, and consequently more easily discernible. The pigment content of the epidermis in white skin is greatest in the skin of the nipple, axilla, scrotum and anus. In the skin of the dark races, all the cells of the basal layer and deeper strata of the rete malpighii contain pigment, while those more superficially placed contain less and less as they approach the stratum granulosum. The melanin granules in the epidermal cells are remarkably uniform in size, and are concentrated towards the upper surface of the cell, forming a cap overlying the nucleus. Pigment-containing cells which possess dendritic processes are also to be seen occasionally in the basal layer—they will be discussed more fully in another part of the paper. Melanin-containing cells exist side by side with melanin-free cells, and apart from their pigment content and occasional dendritic form are indistinguishable from them. In white skin the pigmented basal cells are most numerous on the sides of the rete pegs.

The pigment of the hair is contained in the cells of the matrix, bulb and shaft. In the hair matrix and bulb the pigmented cells consist almost entirely of dendritic forms which are most clearly seen in light hairs, since the dendrites are masked by the presence of much pigment. The pigment of the hair shaft consists of coarse melanin granules situated within and between the cells of the cortex, and in addition the cuboidal cells of the medulla may also contain a certain amount.

Branched melanin-containing cells are normally scattered throughout the dermis, and in addition melanin granules exist free in the interstices of the collagen bundles. The melanin granules within such cells are coarse and irregular in size as contrasted with the fine uniform granules of the epidermal cells. Fusiform melanin-containing cells are also present in the hair papillæ.

Chemical Composition of Melanin.—The figures given by different authors for the percentage composition of melanin vary greatly. Thus from 48 per cent. to 67 per cent. of carbon and from 5 per cent. to 13 per cent. of nitrogen, have been reported. These variations may be explained as due to

varying amounts of impurity, but though the chemical and physical properties of melanin render it difficult or impossible to be sure that a given preparation is pure, the range of variation, when all precautions are taken, seems too great for this. It is more probable that there exists a whole series of chemical compounds, more or less closely related and known to us collectively as melanin. Nevertheless the various analyses, though they differ quantitatively, yield information of considerable value as to the nature of melanin. Carbon, hydrogen, oxygen and nitrogen are invariably present, and of the nitrogen, only a very small amount is liberated by nitrous acid, whence it can be concluded that little or none exists in the form of primary amino groups (NH_2). Iron is absent from the melanin molecule, a fact important as opposing the older view that the pigment is derived from hæmoglobin. There is considerable difference of opinion as to whether or not sulphur forms an integral part of the melanin molecule. It is usually present—amounts up to 7 per cent. have been found—and the existence in the melanin molecule of such substances as hydroxyphenyl cystein has been postulated to account for its occurrence, as also has the participation of glutathione in melanogenesis. Recently, however, Schaaf has succeeded in removing, by purely physical means, the whole of the sulphur of a natural melanin, and it must be concluded that sulphur, when present, constitutes part of an impurity.

Chemical manipulation of melanin has not yielded information of very great value. Oxidation by various agents destroys the colour, reduction yields amorphous and unknown substances. After hydrolysis, amino acids have been isolated, Piettre, for example, having obtained alanine by alkaline hydrolysis, and from 16 grams of melanin hydrolysed by sulphuric acid, 0·1 gram tyrosine, 1·5 grams leucine, and 4·5 grams of an unidentified mixture of amino acids. Though these amino acids may have been derived from some protein impurity in the melanin, it is possible that they actually formed part of the pigment molecule, as will be shown later; and if so, their presence may go far to explain the varying composition of naturally occurring melanin.

Several observers have obtained traces of pyrrole among the breakdown products of melanin, and this was at one time considered to support the view that melanin is formed from

hæmoglobin, of which the prosthetic group is an iron-containing tetra-pyrrole derivative. The method by which the pyrrole is obtained, however, renders such a conclusion, and even the conclusion that melanin actually contains pyrrole, quite unjustifiable, and we really depend on other evidence for our knowledge that pyrrole is present, though condensed with a benzene ring to form indole. The strongest evidence in support of this statement is that adduced by Raper, to be considered later. Besides this, there is the observation by Eppinger (1910) that urine from a case of melanotic sarcoma contained indoles, and that, curiously enough, in the light of more recent knowledge, the amount of these substances was increased by administration of tryptophane, but not of tyrosine.

Briefly, then, we may say that melanin is the name applied to a group of nitrogenous substances, which contain neither iron nor sulphur as an essential part of the molecule, which may contain various amino acids, and which contain the indole ring.

The Chemical Reactions involved in Melanin Formation.—The chemistry of melanogenesis resolves itself into two parts: first, the nature of the source of melanin—the chromogen—together with the reactions by which the chromogen is converted to pigment; and second, the means by which these reactions are brought about in the living cell. It is the first of these parts that is to be considered here.

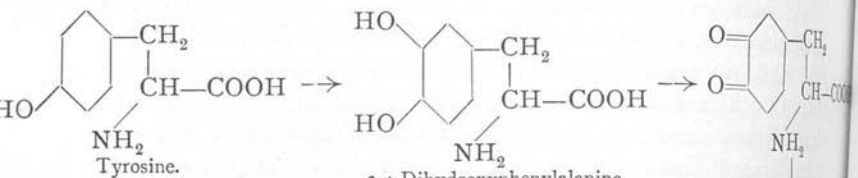
The presence of pyrrole among the decomposition products of melanin has led to the suggestion that a derivative of that substance was to be considered as the source of melanin, and this idea appears to have survived the complete shattering of the old idea that hæmoglobin, with its tetra-pyrrole nucleus, is the parent substance. Thus Rondoni (1920) claimed that the formation of brown pigment followed the treatment of skin sections with dilute solutions of pyrrole, and Quattrini (1923) found deposition of pigment round the site of injection (into the subcutaneous tissues of non-albino rabbits) of α-pyrrole carboxylic acid, of α-methyl indole and of β-ethyl indole. Introzzi (1926) obtained similar results with various pyrrole derivatives in grey rabbits. Recently, however, Peck (1929) has shown that the various substances used by the Italian workers induce pigment formation only in the presence of light, and that, since similar results are obtained by injection of, *e.g.*, acetic acid or by mechanical lesions, they

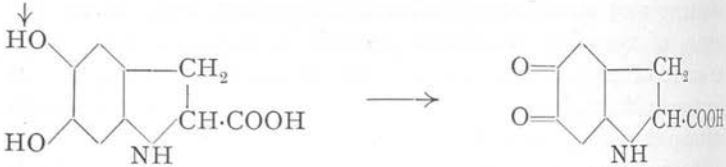
act merely as non-specific irritants and are not chromogens at all.

A considerable number of observations point to the conclusion that the ultimate source of melanin is tyrosine, and that while in some tissues melanin is formed from tyrosine itself, in others only part of the mechanism is present, so that these tissues must be supplied with an intermediate which has been formed from tyrosine elsewhere. As early as 1902, von Furth and Schneider found that tyrosine could act as chromogen in *lepidoptera*, and this observation has been confirmed. Verne (1926) has found tyrosine to be converted to melanin by crustacea, and Schmalfuss and Müller (1927) have extracted dihydroxyphenylalanine, a derivative of tyrosine, from the pigmented wings of certain insects. The most important and convincing evidence, however, both as to the identity of tyrosine with the melanin chromogen and as to the mode of transformation, is that obtained from the study by many workers, including Raper, of tyrosinase in plant tissues and from the study of animal tissues by Bloch (1927).

The earlier work on tyrosinase has often been summarised, and need not detain us here. Tyrosinase is widely distributed in the vegetable kingdom, and catalyses the oxidation of tyrosine by molecular oxygen with production first of a red substance which ordinarily becomes reddish-brown and finally black, the final product being melanin. By careful analysis of the conditions, Raper and Wormal (1923) were able to show that the function of the tyrosinase consisted in the production, from tyrosine, of a red pigment, which in alkaline solution changed spontaneously to a colourless substance. This, in the presence of air alone, was then converted to melanin. Later (1925) the same authors isolated 3-4-dihydroxyphenylalanine from the reacting mixture, showed that with tyrosinase it underwent the same transformations as did tyrosine (but more rapidly), and concluded therefore that it was probably the first intermediate in the conversion of tyrosine to melanin. In 1927 Raper confirmed the position of dihydroxyphenylalanine as an intermediate in melanin formation, and isolated the colourless substance formed from the red pigment by alkali. This substance (isolated actually as a methyl derivative, since it is itself very unstable) proved to be 5-6-dihydroxy indole, and since the corresponding 2-carboxylic acid was also obtained, Raper was able to put forward the following scheme as

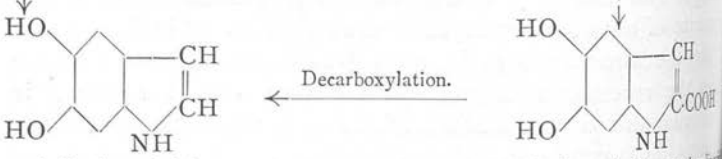
representing the probable course of the earlier stages of melanin formation :—





Auto-reduction and Decarboxylation.

Auto-reduction.



Melanin.

Raper points out that the melanin obtained in this way from tyrosine is not necessarily identical with natural melanin, since tyrosine-containing peptides are acted upon by tyrosinase just as is the free amino acid. It is probable, he considers, that though the initial stages leading to the formation of the indole ring are the same, the peptide linkage persists in the final product. Such a mechanism would account for the production of amino acids by hydrolysis of naturally occurring melanin, and so, as has been mentioned, for some at least of the variations in the composition of melanin.

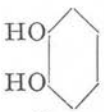
Although in certain forms of animal life a tyrosinase appears to be present, in warm-blooded animals there is no evidence that any mechanism exists for the complete conversion, *in situ*, of tyrosine to melanin. The extensive work on which this statement is based has been summarised by Bloch (1927) and by de Juste (1928). Nevertheless there is probably a similar mechanism at work in plants and in animals, for Bloch has found that though tyrosine is inactive, its derivative, dihydroxyphenylalanine, is converted to melanin in the epidermis, and this substance, it will be remembered, was isolated by Raper as the first product of the action of tyrosinase on tyrosine.

Biological Relationships of Melanin.—Melanin is found almost exclusively in the interior of cells as variously sized granules distributed throughout the cell cytoplasm. From the morphological standpoint it might either be produced as a result of the metabolic activity of the melanin-containing cells or derived from some other source and subsequently transported to these cells. Meirowsky (1909) has shown that melanin production can occur in portions of epidermis which have been cut off from the general circulation and also in Thiersch grafts *in vitro*. These experiments demonstrate that melanin can be formed *in situ* by the cell protoplasm, and that the process is independent of the circulation. This independence of the melanin-forming cell or melanoblast is only relative, since it must ultimately receive its nourishment from the circulation, but Meirowsky's work shows that the final synthesis of melanin from its precursor is a specific function of cellular activity.

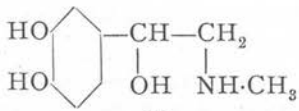
The Mechanism of Melanin Formation.—In the presence of tyrosinase, as has been mentioned, the formation of melanin from tyrosine is apparently due to the oxidative formation of

the red indole quinone through the action of the enzyme. The subsequent reactions, the formation of 3-4-hydroxy indole and its further oxidation to melanin, are able to take place merely in the presence of molecular oxygen, and without the intervention of any enzyme.

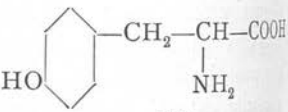
Higher animals are apparently devoid of tyrosinase; and though the chemical reactions are probably similar to those taking place in the presence of tyrosinase, the enzyme mechanism is obviously different. The most notable advance in our knowledge of the mechanism of melanin formation in warm-blooded animals was made by Bloch, whose work arose from a consideration of the facts that in Addison's disease, with dysfunction of the adrenals, there occurs a bronzing of the skin, and that, in cases of generalised melanocarcinosis with melanuria, involving excessive pigment formation, the urine contains excessive amounts of catechol derivatives. The second of these facts indicated the close relationship between the melanin precursor and orthodihydroxy benzene (I), and the first, suggesting a relationship between the precursor and adrenalin (II), suggested that the melanin was formed from some chromogen related to tyrosine (III) but having two hydroxyl groups attached to adjacent carbon atoms in the benzene ring:—



I.



II.



III.

Bloch accordingly tested 3-4-dihydroxyphenylalanine (which, using the initial letters of its German name, he calls "dopa") and found that it gave rise to the formation of melanin in the epidermis. This observation, it should be noted, was made years before dopa was isolated by Raper from the products of the action of tyrosinase on tyrosine.

Sections of skin, soaked in a solution of dopa under appropriate conditions, showed deposition of melanin in just those positions in which pigment formation normally occurs. Moreover, the amount of this response to treatment with dopa corresponded with the known capacity of the skin for melanin formation, being great in skin capable of heavy pigmentation and so small as to be undetectable in skin from albinos or cases of vitiligo. This exact correspondence between

the capacity for pigmentation and the intensity of the dopa reaction, and between the site of melanin formation normally and on treatment with dopa, will be dealt with more fully in a later section of this paper. It is of great importance here, since it affords very strong evidence that the phenomena of the dopa reaction are those of normal melanin formation.

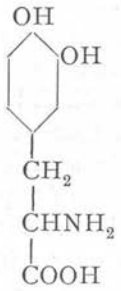
In addition to this deposition of melanin *in situ* Bloch has been able to prepare extracts of skin which, when incubated with dopa, showed a progressive darkening of the solution with, finally, deposition of melanin. Control solutions of dopa without the skin extract remained colourless. As in the case of skin sections, melanin formation was observed only with extracts from skin capable of pigmentation; extracts from the skin of albino animals gave negative results.

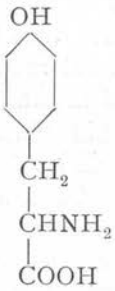
Bloch explains these observations by supposing that those cells which are capable of producing melanin—and only those —contain an oxidising enzyme which he calls *dopa oxidase.* It is incapable of oxidising tyrosine, he thinks, but acts specifically on dopa, converting it to melanin. This theory has been adversely criticised, but a careful examination of Bloch's evidence shows his view to be justified.

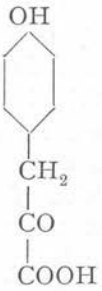
Criticism has been directed against the statement that there exists an enzyme dopa oxidase. It has been suggested by Pzribram (1921) and by de Juste and Verne (1928), for example, that a local alkalinity in the melanoblasts would account for the deposition of melanin there when skin sections are soaked in dopa, since dopa is very readily oxidised in alkaline solution. This suggestion is capable of explaining the negative dopa reaction in cases of albinism, if it be supposed that in these cases the requisite local alkalinity is absent. It is, however, totally incapable of explaining the negative results obtained with *extracts* of albino skin, since in these experiments the *p*H was carefully adjusted to the same value which, with extracts of normal skin, gave positive results.

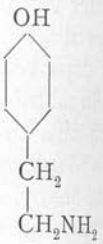
The power of the skin to produce melanin from dopa is destroyed by heat. It is abolished, moreover, by very low concentrations of cyanide, which have no appreciable effect on the *p*H. Some degree of instability to heat is characteristic of enzymes, and though possession of this property is obviously not enough of itself to identify the melanin-forming power of skin as an enzyme, it is certainly suggestive. Still more so is the effect of cyanide, for a great many oxidising enzymes

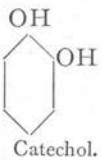
are inactivated by that substance, a fact explained by Warburg as due to the formation of metallic cyanides, the presence of a metal such as iron in certain surfaces constituting the enzyme.

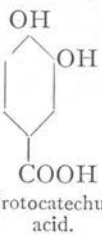

Dopa.


Tyrosine.

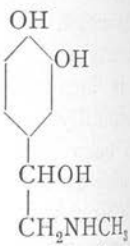

p-hydroxyphenyl-pyruvic acid.

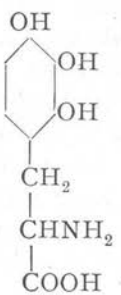

Tyramine.

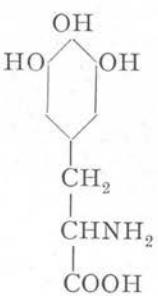

Catechol.

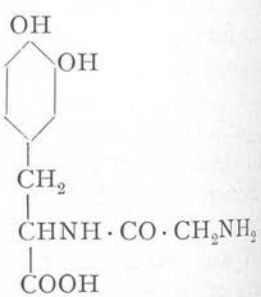

Protocatechuic acid.


3-4-Dihydroxyphenyl acetic acid.


Adrenalin.


2-3-4-Trihydroxyphenylalanine.


3-4-5-


Glycyl-dopa.

It could not be expected that the presence or absence of merely physical conditions of environment would localise the oxidation of one readily oxidisable substance like dopa without behaving similarly towards other equally easily oxidisable substances. Enzymes, on the other hand, are known to be specific, to cause chemical reaction to take place with one

substrate but not with another. In skin, Bloch has shown that whatever it is that causes melanin formation from dopa, it is remarkably specific in its action. He has tested a large number of substances which have been or might be suggested as possible precursors of melanin, or which are chemically related to dopa, all with negative results. A list of some of these substances, with their formulæ, to show their relationship to dopa, is given on p. 506.

The evidence, then, from specificity, from the action of heat, from the behaviour of potassium cyanide (besides that from other directions, such as the behaviour of antiseptics), and from the activity of extracts, points to the existence of a melanin-forming enzyme, Bloch's "dopa oxidase." The occurrence of this oxidase in those cells which are capable of producing melanin, and its absence from all others, indicates strongly that it is the agent responsible for normal melanogenesis. Finally, the production of melanin from dopa, and from no other related substance, is strong evidence that dopa is the actual melanin precursor in the higher animals, and that if in these animals tyrosine is the ultimate source, it must undergo the first stage of oxidation—to dopa—elsewhere than in the melanoblasts.

Technique of Dopa Reaction.—In actually carrying out the dopa reaction, it is essential that the water used in making up the solutions should be pure and sterile, and that the *p*H of the dopa solution should be carefully adjusted to the optimum. Successful results are obtained only within a very narrow range of *p*H; with solutions too acid the reaction is completely negative; with solutions too alkaline the dopa oxidises spontaneously and a general diffuse staining of the sections results.

The pure water is prepared by the following series of distillations, is stored in Jena glass flasks, and boiled immediately before use. Jena glass apparatus should be used for the distillation apparatus. Ordinary distilled water is

(1) distilled with potassium permanganate (2 grams per litre);
(2) this distillate is distilled with phosphoric acid (4 grams 80 per cent. H_3PO_4 per litre);
(3) this distillate is distilled with sodium carbonate (30 grams Na_2CO_3 per litre);
(4) this distillate is distilled alone.

For the adjustment of the *p*H, a phosphate buffer is used, and it is prepared by mixing in a certain proportion solutions of (A) potassium dihydrogen phosphate (9·078 grams KH_2PO_4 in 1000 c.c.) and (B) disodium hydrogen phosphate (11·876 grams $Na_2HPO_4 2H_2O$ in 1000 c.c.). One drop of buffer solution so prepared is added to each c.c. of dopa solution, the latter containing 1 mgm. dopa (obtainable from Messrs Hoffmann la Roche) per c.c. The *p*H of the mixture must lie between 7·3 and 7·4, the optimum being 7·34. The simplest method of finding the correct mixture of phosphate solutions to give this optimum reaction is that of trial and error. A series of mixtures is prepared by adding to one part of solution A amounts of solution B ranging from four to ten parts. The seven buffer solutions so obtained are tested by preparing from them seven dopa solutions.

The dopa solutions are incubated with a number of skin sections for twenty-four hours at ordinary room temperature or for three hours at 37° C. The sections are then mounted and examined. That set of sections which shows the strongest dopa staining with the least general darkening of the section has been produced under the optimum conditions, and the phosphate mixture used in its preparation is taken as standard for future work. Fresh reagents, of course, must be standardised in the same way, and, if necessary, a wider range of buffer mixtures must be tried. The present authors have found a mixture of one part of A and four parts of B to give satisfactory results.

Findings with Dopa.—When frozen sections of human skin are left in contact with dopa solution certain cells of the basal layer of the epidermis and hair follicles and the hair matrix become darkened. This darkening is due to the deposition of fine black or brown granules in the cell cytoplasm and constitutes a positive dopa reaction. The final appearance resulting from this reaction is indistinguishable microscopically from that of normal melanin pigmentation. The blackening is confined to the cell cytoplasm and does not involve the nucleus, thus affording additional evidence against the previously held theory of the immediate participation of the nucleus in the process of melanin formation. If the reaction is strongly positive, the whole cell cytoplasm is a diffuse black and the granules seen in less strongly reacting cells are masked. The intensity of the reaction coincides with the existing degree of pigmentation present in the portion of skin examined or, in

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FIG. 1.—Pigment-containing cells situated in the basal layer of the epidermis, stained with silver (× 1000). Note the supra-nuclear concentration of pigment granules (AA).

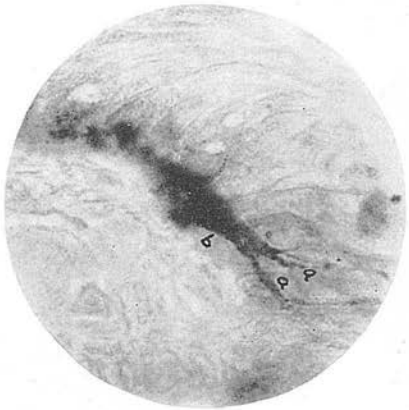

FIG. 2.—Dopa positive dendritic cell in the basal layer of the epidermis. The dendrites (aa) leave the cell body (b) and pass laterally and then upwards betwixt the adjacent cells of the basal layer.

FIG. 3.—Dendritic cell stained with silver (× 800). The cell body lies in the basal layer of the epidermis and the processes pass laterally and upwards amongst the adjacent basal and prickle cells.

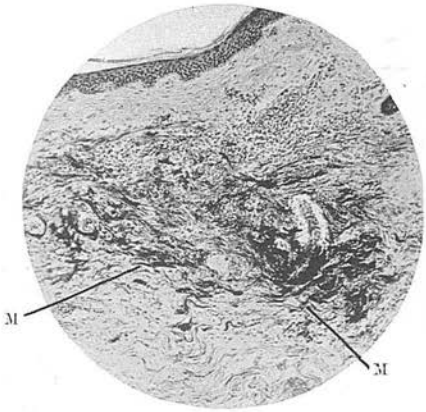

FIG. 4.—Dopa stained section of "blue nævus" (× 100). Note ribbon-like masses of dopa positive melanoblasts (MM) in the middle dermis.

other words, a close parallelism exists between natural pigmentation (actual or potential) of a skin and its capacity to oxidise dopa. This parallelism affords strong presumptive evidence that the reaction is identical or at least closely allied to normal melanogenesis, that it takes place in the epidermal melanoblasts, and that it indicates the presence of the melanin-producing ferment within these cells.

Dendritic Cells.—These epidermal melanoblasts are morphologically of two types, the one resembling in appearance the adjacent dopa negative non-pigmented basal cells and the other possessing dendrites. The dopa positive dendritic cells are situated in the basal layer in the epidermis and hair follicle and in the hair matrix, and are in contact with the basal cells in these situations. In highly pigmented skin they are also to be found in the lower strata of the rete mucosum. The cell body, while varying considerably in size, is larger than the ordinary basal cell and may be globular, fusiform or angled. Narrow filamentous dendritic processes arise from all the surfaces of the cell body to extend laterally and upwards amongst the basal cells and cells of the rete. These processes are, as a rule, tortuous, of varying length, and branch repeatedly. They never pass downwards into the dermis, where dopa positive cells are not normally found. Dendritic cells are most numerous in skin which has been subjected to ultra-violet rays, radium, X-ray and thorium X irradiation, and in acanthotic processes. With the dopa reaction the dendritic cells stain a diffuse black, and only rarely show the presence of black or brown granules. In addition to giving a positive dopa reaction, they contain varying amounts of formed melanin, but the dendritic form is probably only seen with silver or dopa staining (Figs. 2 and 3).

Such dendritic cells have long been known to occur in amphibia, in the lower mammals, and in the skin of man, and have been studied extensively in connection with embryological pigmentation. Following the discovery of the dopa reaction, the pigmented dendritic cells have been reinvestigated, and much additional knowledge as to their morphology and biological relationships has been acquired. They have been especially studied in the human skin, and their function and significance is now more fully understood.

Melanin-forming dendritic cells are quite distinct from the branched cells of Langerhans, which also occur in the epidermis

and which are nerve elements. Comparative studies by Bloch, who used a special gold impregnation method in conjunction with the dopa reaction, have conclusively demonstrated this difference, although it had previously been held that the Langerhans cells and the pigmented dendritic cells were identical. It is almost certain that the pigmented dopa positive dendritic cells are developed from the normal basal cells, and that they represent an active phase in the pigment-forming function of the latter. Transition forms between dopa positive basal cells and fully-formed dendritic cells can be traced in highly pigmented skin, and it would appear that each and every basal cell has the capacity of assuming the dendritic form when the skin is subjected to a pigment-forming stimulus. Following the cessation of such a stimulus, the dendritric cells resume the basal cell form and their number thus diminishes. The transition forms are represented by basal cells which possess rudimentary dendritic processes and dendritic cells have not been observed to undergo mitotic changes.

Becker (1927) found dendritic cells in all the regions of the cutaneous surface which he studied and in the mucous membrane of the mouth and pharynx. The relative proportion of dendritic to non-dendritic cells was found to vary in different regions. The dendritic cells predominated in the pharynx and were present in the smallest proportion in the epidermis of the nipple and abdomen. A high degree of pigmentation is not, therefore, necessarily accompanied by a high percentage of pigmented dendritic cells, and this fact supports the view that the dendritic form is associated with periods of active pigment production within the cell. From their universal distribution it may be concluded that they are normal cellular constituents of the epidermis.

The structural appearance of the dendritic cell has led to the suggestion that the dendrites constitute a distributing mechanism whereby the pigment formed within the cell is handed on to adjacent non-pigmented epidermal cells. Pautrier (1928) and his co-workers claim that the dendritic cells form a connecting link in a nutritive syncytial system operating between the dermis and epidermis, and that they obtain the chromogen through cellular connections in the dermis, and subsequently distribute fully-formed melanin to the epidermal and dermal cells. Against this attractive theory of the function

Fig. 5.—Dopa stain (×500) showing dopa positive m dermis.

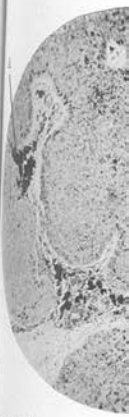

Fig. 7.—Section of ben with silver (×200). and in the interior of stroma. B, melanin cells. Compare with the same tumour.

Fig. 5.—Dopa stained section of "blue nævus" (×500) showing fusiform and elongated dopa positive melanoblasts (MM) in the dermis.

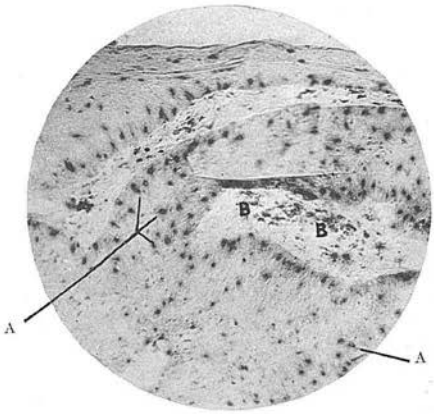

FIG. 6.—Dopa stained section of benign melanoma. AA, dopa positive cells. BB, formed melanin lying free or within chromatophores in the stroma of the tumour; there is no darkening of the chromatophores with dopa. Compare with silver stained section of the same tumour.

Fig. 7.—Section of benign melanoma stained with silver (×200). A, melanin lying free and in the interior of chromatophores in the stroma. B, melanin granules in melanoma cells. Compare with dopa stained section of the same tumour.

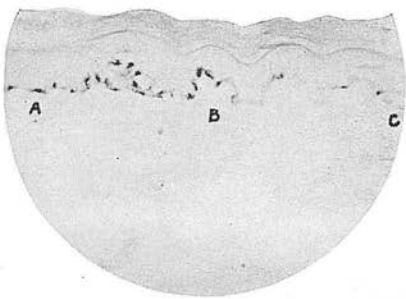

FIG. 8.—Dopa stained section of the edge of a patch of vitiligo (×100). AB, hyperpigmented border showing dopa positive cells in the basal layer. BC, depigmented area showing an almost negative dopa reaction. Compare with silver stained section of the opposite border of the same patch.

of the dendritic cell it must be stated that most authorities, including Bloch, have failed to observe the dendritic processes passing into the dermis.

Dermal Pigmentation.—The branched and fusiform pigmented cells which are to be found scattered throughout the dermis do not give a positive dopa reaction, and therefore may be regarded as chromatophores which are incapable of melanin formation. By injecting melanin into the dermis Miescher (1922) has shown that the introduced pigment is phagocyted by the connective tissue cells, which are then indistinguishable from true chromatophores, and it would therefore appear that the latter derive their pigment from the epidermis. In contradistinction to these phagocytic dopa negative chromatophores, which are always to be found in the dermis, true melanoblasts are occasionally met with in this situation. They occur exclusively in the pigmented patches of blue nævi and Mongolian spots. The latter are present in the sacral region in white races at birth, but are soon lost. In both conditions the pigmented cells are deeply situated in the dermis, are ribbon like, and arranged in groups. They give a positive dopa reaction and are therefore capable of melanin formation (Figs. 4 and 5). Similar melanoblasts are found in the connective tissue of the iris and ciliary body, in the meninges, and in the dermis in monkeys, mice, negro-fowls and other animals. Apart from these situations melanoblasts are unquestionably derived from ectodermal tissue, but on account of the fact that they can occur in the dermis the question of their possible mesodermal origin at once arises. Bloch considers that those melanoblasts which are found in the dermis are mesodermal cells, in spite of the fact that the dopa reaction is positive and that, apart from the isolated examples under consideration, the power to oxidise dopa specifically would seem to be an exclusive property of ectodermal tissue. Dawson (1925), on the other hand, was of the opinion that the pigmented cells of the dermis were either cells which had migrated from the epidermis or connective tissue cells which had engulfed pigment granules. In the absence of dopa reaction he was unable to distinguish between dermal chromatophores and melanoblasts. Such a migration into the dermis of epidermal melanoblasts has been followed histologically in embryonic amphibian and mammalian skin, notably in the dog's snout and ear. It has also been observed by Spencer (1923) in the

ink-sac of the cuttle-fish and in the eyeball. Hortega (1921), on the other hand, believed that the epidermal situation of melanin was secondary, and that the pigment had originally been formed by mesodermal cells which had subsequently migrated into the epidermis. This view, which was shared by several other workers, was based on histological observations in embryo rabbits. Peck (personal communication) has recently repeated this work, however, and has shown, by means of reconstructed serial sections, that the pigmented dermal cells observed by Hortega were in reality epidermal melanoblasts situated on the sides of the hair follicles. These purely morphological findings, taken in conjunction with the positive dopa reaction, afford strong presumptive evidence that the dermic melanoblasts were originally derived from ectodermal tissue, in spite of their ultimate complete separation from its neighbourhood.

The Pigment of Hair.—The pigment-containing cells of the hair matrix and bulb give a positive dopa reaction, but the pigmented cells of the shaft and papilla are dopa negative. These results show that the pigment is formed in the matrix and bulb, the fully-formed melanin then passing up into the hair shaft in cells which have lost their pigment-forming capacity. A small amount of pigment migrates into the dermis and is phagocyted by the connective tissue cells of the papilla, which then become chromatophores. The transference of pigment from matrix cells to dermis is augmented in those states which are characterised by pigmentary alterations—for example vitiligo, canities and following X-ray irradiation—and under these conditions the chromatophores of the papillæ are greatly increased in number. In the human fœtus pigment first appears in, and is for some time situated exclusively in, the hair matrix about the fifth month. As the hair whitens with age there is a progressive diminution in the pigment production of the matrix cells and a coincident weakening of the dopa reaction, which finally disappears altogether. This phenomenon is due, not to any alteration in the existing pigment of the hair shaft, but to the absence of the melanin-forming ferment in the matrix cells and a consequent cessation of pigment formation. The process of whitening is therefore a gradual one, and it is difficult to understand the mechanism involved in cases which are reported to have suddenly developed white hair as a result usually of nervous shock. To explain

this phenomenon it has been suggested that air-bubbles are suddenly formed throughout the hair shaft, with the result that any contained pigment is masked by a physical effect. In animals which assume a light coat in winter, such as the mountain hare and ermine, there is a seasonal variation in the pigmentary activity of the hair matrix, and this function may even undergo a temporary complete cessation. In some dark-haired animals the cells of the hair matrix and bulb alone contain pigment, the epidermal cells being devoid of it. For this reason the skin of these animals is white or has a bluish tinge, due to the deeply-situated melanin shining through the translucent overlying epidermis.

Fate of formed Melanin in the Skin.—The bulk of the melanin which is formed in the epidermis is carried upwards towards the surface and ultimately extruded along with the fine powdery desquamation of the stratum corneum. A certain amount finds its way from the basal layer into the dermis, where it is phagocyted, or remains in the connective tissue spaces, and is ultimately removed in the lymph stream. This dermic transportation of pigment is greater when regressive or degenerative changes are taking place in the epidermis or hair matrix, and is more in evidence following radiations which have produced some degree of epidermal destruction in addition to pigmentation, and also in the normal atrophy and whitening of hair which accompanies advancing years.

The Effect of the Leucocytes on Dopa.—In addition to the melanoblasts, the leucocytes are capable of oxidising dopa in virtue of their contained polyphenolase. This is an entirely non-specific reaction, and can be obtained under conditions entirely unfavourable to the occurrence of the specific dopa reaction.

Pigment of the Retina.—From the standpoint of pigmentary function, the retina presents an interesting phenomenon. In embryonic life the pigmented cells give a positive dopa reaction and are actively engaged in pigment formation. When full development is reached, these cells lose their power to react with dopa, and active pigment production has then ceased to take place. Such a loss of melanogenic power is obviously to be desired in the retina, for when a full complement of pigment has been acquired by the cells, any subsequent fluctuation in this amount would seriously interfere with visual function.

Summary of the Dopa Reaction.—In the foregoing account it has been emphasised that there is convincing evidence of a

histological and biological nature to show that the dopa reaction resembles very closely or is identical with the normal process of melanin formation. The dopa reaction demonstrates the presence within the cell of an oxidase, the action of which seems so far to be specific. Although the existing evidence is strongly in favour of the absolute specificity of this ferment, and of its identity with the melanin-forming ferment, there is as yet no definite proof of this. It is certain, however, that the ferment exists in the melanoblasts, and that any cell which can be shown to contain it may be regarded as capable of producing melanin. On the other hand its absence from a pigmented or non-pigmented cell may be taken as evidence of the temporary or permanent absence of pigmentary function in that cell. It is for these reasons, apart from chemical considerations, that the dopa reaction is such an invaluable adjunct to the study of the problem of melanogenesis.

The results obtained with the dopa reaction show that melanin formation is almost entirely a function of tissue derived from the ectoderm, and of the skin in particular. It is highly probable that melanogenesis occurs exclusively in tissue of ectodermal origin, and although melanoblasts are occasionally found in other situations, there is a good deal of embryological evidence which suggests an ultimate ectodermal derivation for such cells.

Pathological Melanin Pigmentation—(1) *Addison's Disease.* —Any theory of melanin formation must offer a satisfactory explanation for disturbances of pigment metabolism which occur in disease, and such an explanation must be consistent with the facts observed. From the pathological standpoint Addison's disease offers an admirable test. Here the activity of the suprarenal glands is much impaired, and a general hyperpigmentation of the skin is one of the cardinal symptoms of their deranged function. The hyperpigmentation can be explained in the following way according to the theory of Bloch. On account of the failure of the adrenal glands to synthesise adrenaline from its mother substance, an excess of this normal precursor circulates in the blood. The close chemical relationship between adrenaline and dopa, and the possible identity of the latter with the precursor of adrenaline, has already been commented on. It is thus possible that the excess of adrenaline precursor, on reaching the skin cells, is acted on by the contained melanin-forming ferment and

transformed into melanin. The results with the dopa reaction in the skin in Addison's disease are consistent with this hypothesis, and it is found that the reaction is weak, although the cells contain an excess of melanin. This is explained as being due to exhaustion of the ferment content of the cells, resulting from an excessive supply of chromogen. Cells throughout the entire rete, and even the stratum corneum, may contain melanin according to the intensity of the disease, but very few dendritic forms are found. In the presence of normally functioning adrenal glands this excess of chromogen would have been converted to adrenaline, and the formation of melanin by the skin may here be interpreted as an excretory function. This explanation is simple, and will bear scrutiny from a chemical and pathological standpoint. It is thus a strong argument in favour of the theory that melanogenesis consists essentially in the action of a specific ferment on an aromatic amino acid, although it brings forward no further evidence for the identity of the chromogen with dopa.

The hyperpigmentation which frequently accompanies advanced malignant neoplastic disease is possibly due to a secondary diminution in the functional activity of the supra-renal glands, in which case its immediate mode of production would be analogous to that suggested in Addison's disease.

(2) *Melanòma.*—In melanomata the infiltrating "nævus" cells contain more or less pigment, or pigment may be entirely absent from them. Melanin granules almost invariably exist free or contained within branched cells in the stroma of the tumour. The dopa reaction is positive in all or many of the cells throughout such a tumour, and it demonstrates the presence of a capacity for pigment production in non-pigmented as well as pigmented cells. It is negative in the pigmented cells of the stroma, which are thus shown to be chromatophores The dopa reaction further demonstrates the fact that many of the melanoma cells are dendritic, and in faintly reacting tumours these dendritic and non-dendritic dopa positive cells have a tendency to occupy the periphery of the infiltrating masses (Figs. 6 and 7). The fact that the melanoma cells give a positive dopa reaction is additional proof of a biological nature that the melanoma cell is epidermal in origin, since the existence of mesodermal melanoblasts is at least doubtful.

(3) *Albinism.*—In complete albinism there is total absence of melanin pigmentation in the skin and hair, and the dopa

reaction in these situations is negative. It is significant that the skin of albinos responds to ultra-violet irradiation by vasodilatation, but no pigment is formed. This is a naked-eye demonstration that pigment formation is immediately independent of the circulation, and suggests that it is an autochthonous function of the epidermal cells.

(4) *Vitiligo.* — In vitiligo there is complete absence of pigment from a small area of skin, which is surrounded by an area of hyperpigmentation. The dopa reaction is negative in the epidermal cells of the depigmented area and strongly positive in the surrounding zone of hyperpigmentation (Figs. 8 and 9). A similar condition is found to exist in the skin of piebald animals.

(5) *Inflammatory Processes.*—The dopa reaction is frequently lost in areas of skin inflammation, indicating a temporary loss of pigment function, or rather a diminution in ferment activity. Following this period the reaction becomes strongly positive, and may be associated with hyperpigmentation. The reason for this is obscure, but the immediate cause is apparently some interference with cell metabolism consequent on the inflammation. This association of hyperpigmentation and strong dopa reaction with inflammation is especially well marked in lichen planus. Peck (1929) has shown experimentally that the hyperpigmentation which is associated with inflammation can take place in the complete absence of light.

Function.—Skin pigmentation fulfils a more limited function in man than in the lower vertebrates and invertebrates. Its universal rôle is to protect the organism against the injurious effects of the solar spectrum. In man its activity is confined to the performance of this function, but in the lower forms of life variations in colour, both pigmental and structural, play an important part in the preservation and propagation of the species. Melanin is the only normally occurring pigment in the human race, and the one which is most widely distributed in the animal kingdom, where it even plays a large part in the production and variation in shade of structural colours.

The position of melanin in human epidermal cells, forming a cap covering the upper surface of the nucleus, suggests that it is so situated in order to protect the vital part of the cell from some external trauma. The amount of pigmentation corresponds to the protective requirements of the race against actinic rays, and in the complete absence of pigment the skin

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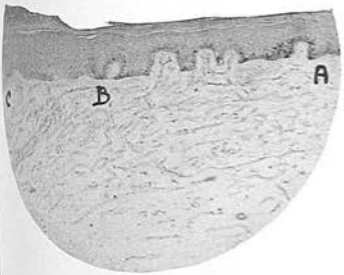

Fig. 9.—Section of the edge of a patch of vitiligo stained with silver nitrate to demonstrate formed melanin (× 100). CB, vitiliginous area showing complete absence of formed melanin in the basal layer. BA, hyperpigmented border showing abundant melanin within the basal cells. Compare with Fig. 8, which shows a dopa stained section of the opposite border of the patch.

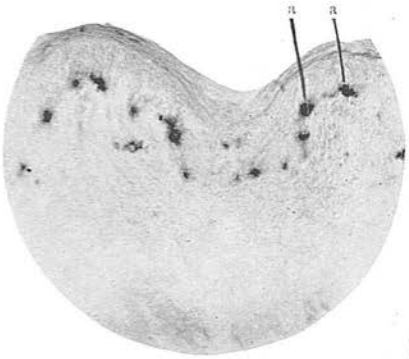

Fig. 10.—Dopa reaction in normal skin (× 250) showing dopa positive cells (aa) in the basal layer of the epidermis. Compare with Fig. 11, which shows the same skin four days after ultra-violet radiation.

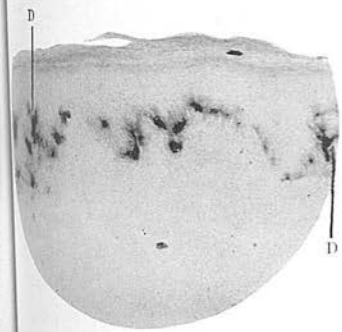

Fig. 11.—Dopa reaction in irradiated skin four days after radiation with ultra-violet rays (× 250). Compare with Fig. 10, which shows the same skin prior to irradiation. Note the increase in the number of dopa positive cells and the appearance of dendritic forms (D) as a result of irradiation.

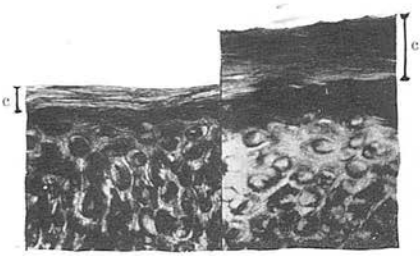

Fig. 12.—To illustrate the keratinisation response of the epidermis to ultra-violet radiation. To the left is the epidermis before radiation, to the right is the closely adjacent epidermis six days after radiation. Note the three-fold increase in thickness of the stratum corneum (cc). (Both × 380.)

is extremely sensitive to the injurious effects of this form of energy. Melanin has the property of absorbing very completely the shorter wave-lengths of the spectrum. In the skin of dark-coloured races the distribution of melanin, which is to be found as high as the stratum granulosum, and even in the stratum corneum, affords an admirable protection for the whole epidermis and underlying structures against these wave-lengths. Since this function is one of extreme importance to these races, the pigment content of the skin is maintained at a constant level. On the other hand, in a large proportion of the fair races not only does the melanin content of the skin undergo an obvious seasonal variation in the exposed parts, but the pigment is situated exclusively in the basal layer of the epidermis. In this situation it can afford no protection for the overlying rete cells, which are thus exposed to the full intensity of the actinic spectrum. The pigmentation which follows radiation of the skin is produced by the actinic rays, the spectrum having a wave-length between 2900 A.U. and 3300 A.U. The first effect of radiation is to produce an immediate but transient diffuse bright red erythema, which is due to the heat rays. After a short latent period, the length of which depends on the intensity of the exposure, a second erythma appears, which is due to the actinic rays and which is strictly limited to the site of application. This second erythma persists for several days, and is accompanied sooner or later by pigmentation and desquamation. Pigmentation is not evident for a day or two following radiation, and after its first appearance it increases in intensity for a short time. It is not a necessary accompaniment of the radiation erythema, a fact which is well illustrated in albino skin, which reddens but does not pigment after radiation. On the contrary, pigmentation without a preceding erythema does not occur. The pigmentation is strictly limited to the area on which the actinic rays have acted, and the pigmented area is bounded by a clearly defined margin. Such naked-eye observations point to the local and independent cellular formation of melanin. Melanin pigmentation can be produced in the absence of light stimulation, as is the case when a small area of pigmentation is left to mark the site of an artificially produced blister from which light has been purposely excluded. In this case the inflammatory reaction has evidently produced a state of temporary hyperactivity in the pigment-forming basal cells.

A similar phenomenon has been noted in connection with inflammatory skin lesions situated on covered regions.

In the epidermis of white races, two separate protective reactions are provoked by ultra-violet irradiation, and also by irradiation with thorium X. Using the latter form of energy, Peck (1930) has shown that it causes an immediate increase in the number of dopa positive basal cells and that the proportion of dendritic to non-dendritic cells increases until almost every dopa positive cell is a dendritic cell (Figs. 10 and 11). Following closely on this increase in the intensity of the dopa reaction there is an increase in the amount of fully-formed melanin. The increase in the intensity of the dopa reaction is thus the first step in the mechanism of pigment formation. Later the number of dopa positive cells diminishes until a dopa reaction of normal intensity and a normal proportion of dendritic to non-dendritic dopa positive cells is reached. Shortly after this reduction in the dopa reaction the melanin content of the skin returns to normal. Obviously the effect of the irradiation has been to stimulate the activity of the dopa-forming ferment in the basal cells or to produce an optimum intracellular environment for the formation of melanin. This parallelism between the dopa reaction and melanin production following a pigment-forming stimulus is extremely important evidence in favour of the specificity of the reaction and its actual or close identity with the normal process of melanogenesis. The present authors have shown that similar changes in the dopa reaction and melanin content of the skin take place as a result of ultra-violet irradiation.

This increase in the melanin content of the basal layer can only act as a protection for the structures in the dermis, since, apart from scattered pigment granules in the lowest strata of the rete malpighii, no melanin is found in this layer. Peacock (1925) considers that the fluorescent action of the stratum corneum constitutes an immediate protection against the action of ultra-violet rays, since a proportion of the incident rays could thus be dissipated at the surface as light. He regards pigmentation as a later stage of the same protective process.

Miescher (1929) has pointed out that the sensitivity of white skin to ultra-violet irradiation varies in different regions of the body, according to the thickness of the stratum corneum. Thus the palms and soles are infinitely less sensitive than the flexor surface of the forearm, which in turn will tolerate a more

intense exposure to ultra-violet rays than the conjunctiva. It has been shown experimentally that the effect of ultra-violet irradiation is not only to increase the amount of melanin in the cells of the basal layer, but also to increase the thickness of the stratum corneum and so form a protective layer for the subjacent non-pigmented rete malpighii (Fig. 12). This mechanism is more easily and quickly brought into play than the increase in pigmentation, and by this means a skin can become tolerant to actinic rays before pigmentation has had time to develop, or even in its absence.

The efficiency of this mechanism depends on the absorptive power of the stratum corneum for actinic rays. Freund showed that the epidermis from blisters absorbed everything shorter than 3250 A.U., and that hardened skin scales absorbed all wave-lengths shorter than 3440 A.U. when yellow and shorter than 3290 A.U. when colourless. Clark concludes that a layer of epidermis 0·1 mm. thick will absorb practically all wave-lengths shorter than 3000 A.U. The thickness of the stratum corneum varies greatly over the body surface, measuring approximately from 0·007 to 0·02 mm. in the skin of the abdomen, thighs and anterior surface of the forearm, from 0·03 to 0·05 mm. on the back of the hand and foot, and from 0·1 to 0·5 mm. on the palms and soles. The sensitivity of the skin in these regions to actinic radiation corresponds closely to the prevailing thickness of the stratum corneum. More precise determinations of the absorption curves of actinic rays by the various layers of the skin have recently been made by Bachem (1929). This author finds that the stratum corneum is very transparent in the visible and near ultra-violet, but at 3000 A.U. a strong absorption band starts, with a maximum at 2800 A.U. The stratum granulosum has a curve which is very similar to that of the stratum corneum, but with greater absorption coefficients, particularly in the visible. The stratum germinativum is the most transparent, while the corium has a large absorption coefficient in the visible. From this it is obvious that the various layers differ considerably in their capacity to absorb light.

In view of the fact that the anti-rachitic effect is greatest at 2800 A.U., and that there is a sensitivity minimum for erythema at the same wave-length, Bachem suggests that vitamin activation takes place in the stratum corneum and that "the erythema originates in the stratum germinativum or corium." It is thus possible to explain the fact that while wave-lengths less than

2900 A.U. may cause violent skin inflammation, they do not produce much pigmentation, since they are to a great extent absorbed before they can reach the pigment-forming basal layer of the epidermis.

The presence of melanin in the epidermis has several effects on the incident actinic rays. It converts the skin from a white reflecting surface into a dark absorbing one, and thus assists in the absorption of energy. The pigment acts as a screen for the protection of the deeper structures, and the absorbed light is transformed into heat. Heat absorption, heat production and heat radiation are increased in this way, and a dark skin warms more quickly and thoroughly than a fair skin and holds more heat. Chemical energy is thus converted into heat, which is easily dissipated by radiation from the body surface, and as the absorbed heat stimulates the sweat glands a further surface-cooling effect is produced by the evaporation of their secretion.

It has been held that the increase in the melanogenic function of the skin is closely bound up with the curative effects of ultra-violet radiation therapy in certain diseases. It is now generally admitted that the production of pigmentation is not a necessary accompaniment of improvement or cure in such cases, and the presence or absence of pigmentation must be regarded as of doubtful prognostic importance. Ultra-violet radiation of the skin stimulates other biological processes besides that of pigment formation, but there is no evidence of a close relationship between melanogenesis and immunity phenomena.

The effect of changes in temperature on melanogenesis has already been commented on in connection with those animals which show a seasonal variation in the depth of colour of their fur. A striking example of the influence of temperature on melanin production is met with in the Himalayan rabbit. This animal is white except for the snout, tips of the ears, and paws, which are black. If this animal is kept in low temperatures the markings do not change, but if under the same conditions an area of white fur on the back is shaved, the fur which grows in is temporarily black. In this case cold has produced the opposite effect to that seen in Arctic animals, and no satisfactory explanation of the phenomenon has as yet been suggested.

A considerable length of time is required for changes in the melanin content of the human skin to take place, and any

such change involves either a new formation of melanin or the absorption of that already existing. In certain amphibians, reptiles and fishes, however, alterations in the distribution of melanin, and consequent changes in the general skin markings, can occur very rapidly. Such changes are brought about through the agency of contractile melanin-containing dendritic chromatophores situated in the dermis. These cells are under the influence of the nervous system and are subject to reflex stimuli through the special senses, and to the action of the endocrine secretions and the emotions. By their expansion the general skin colour becomes darker, while spotting and a lighter colour is produced by their contraction. By means of this mechanism a protective mimicry of their surroundings can be rapidly produced and varied according to changing requirements of the environment. A similar, but much more slowly produced, protective colour change is observed in those pigmented animals which produce white fur in the winter.

The melanin content of the pigmented layer of the retina is of the utmost importance to the reception of a clear visual image, and for this reason the pigment content of the retinal cells is constant. Corresponding to this the dopa reaction is only present in these cells during embryonic life, and has disappeared before the assumption of visual function, by which time the retina has acquired its full complement of melanin.

In man and mammals, the small amount of melanin found in the central nervous system and meninges is probably the worthless vestigeal relic of a previously well-developed pigment system which acted as a protection for the central nervous system. It is possible, as Spence has suggested, that aberrant dermic melanoblasts have originally been developed in this situation, and have been carried by the growing nerves to distant situations.

Control of Pigment Metabolism.—The most obvious factor controlling melanogenesis in man is the external stimulation of the actinic rays of the sun. Although racial pigmentation is a hereditary characteristic, original differentiation must have been due, to a large extent, to the reaction of the tissues to this stimulus in an attempt to adapt themselves to their environment. In the skin of the dark races, the inheritance of a high pigment content affords a maximum degree of protection against these factors, which shortly after birth cease to influence the degree of skin pigmentation. At birth, however, such skin is not

excessively pigmented, and only acquires its full complement of melanin after exposure to light. When pigmentation is once fully developed, it subsequently remains at a very constant level.

The influence of the endocrine secretions on melanogenesis, although of great importance, is as yet little understood. That these hormones can produce a profound effect on pigmentation is clearly seen in the various disease processes which involve the respective ductless glands, and which are accompanied by alterations in pigment metabolism. Such pigment anomalies are common to all types of endocrine dysfunction, although derangement of the adrenal glands is accompanied by the most marked disturbance. From such pathological evidence as is available, however, no definite controlling action on melanogenesis can be assigned to any one particular endocrine gland. The connection between the female reproductive organs and melanin formation is illustrated by the increase in skin pigmentation which so frequently occurs during human pregnancy. Becker has found that this increase in the melanin content of the skin is associated with a relative increase in the number of dendritic cells. The increase is essentially an exaggeration of a normal physiological process, and denotes the existence of a close functional relationship between these two systems. In animals and birds, the gonads and thyroid gland exert a definite influence on melanin pigmentation, and to their secretions is due the pigmental differences which distinguish the sexes. As has already been pointed out, the central nervous system plays an important part in the ever-changing distribution of the skin pigment which occurs in the lower vertebrates. Such variations are not, however, due to alterations in the rate of melanin formation, but are brought about by changes in the surface area of cells containing fully-formed melanin.

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A STUDY OF THE SKIN VESSELS IN SOME FORMS OF INFLAMMATION OF THE SKIN

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The responses of the blood vessels of the human skin to histamine were analysed by Lewis (1), who concluded that the action of this substance on these vessels could be separated into the following three parts: (*a*) Active local vaso-dilatation and (*b*) increased permeability of the minute endothelial vessels, both of which are brought about by the direct action of the drug on the vessels themselves and (*c*) dilatation of the arterioles, due to a local nervous reflex, which results in a widespread passive dilatation of the associated minute vessels which have not already been under the direct influence of the drug.

These three effects constitute the "triple response" which takes the visible form of local redness, and oedema, with a surrounding red flare.

Mechanical, electrical, thermal, and chemical injury all produce essentially the same responses of the skin vessels (triple response). The observations of Lewis show conclusively that in all such types of skin injury the vascular response is brought about by the action of a chemical substance liberated in the tissue spaces as a result of the injury. He argues that "any reaction conforming in the details to the type reactions described, and reasonably explainable in terms of cellular damage, is of the same fundamental kind." (p. 90) He concludes by induction that "whenever the skin displays the prompt triple response here described, this triple response is due, irrespective of the circumstances in which it

[1] In receipt of a part time grant from the Medical Research Council.

and oedematous, then, when the oedema has subsided but deep redness persists, the skin remains for some while refactory, in the absolute or relative sense, to a repetition of the same stimulus, or to any stimulus that produces a similar original lesion on control skin." (p. 242) If, however the skin has been incompletely reddened by the first stimulus then refractoriness to the second stimulus is usually incomplete. Refractoriness only affects the permeability of the vessels.

When the minute vessels are actively dilated as the result of injury or histamine puncture, they are temporarily irresponsive, in the absolute or relative sense to vaso-constrictor substance (adrenalin and pituitary extract). This does not apply to the passively dilated minute vessels responsible for the flare.

Bier's spots refer to the dead white areas which develop on the skin of a limb in which vascular congestion followed by complete occlusion of the circulation has been brought about. These white areas are due to an active constriction of the minute vessels. This vaso-constriction is held by Lewis to result from the formation of vaso-constrictor substances in the tissues spaces, while it is attributed by Rous and Gilding to heightened irritability of the vessel walls.

REACTIONS OBSERVED IN SKIN LESIONS

The authors investigated the following types of skin lesions: (*a*) One case of generalised exfoliative dermatitis; (*b*) four cases of psoriasis; (*c*) one case of tinea corporis; (*d*) one case of traumatic dermatitis; (*e*) two cases of severe erythema due to ultra-violet radiation; (*f*) one case of erythema due to the application of a mustard plaster. The erythematous lesions in these cases were treated with 1:10,000 histamine puncture, 1:1,000 adrenaline puncture, and when possible with congestion and circulatory arrest for Bier's spots. If the dilatation of the minute vessels responsible for the initial redness was due to the presence of a histamine-like substance in the tissue spaces it might be expected that the lesions would show either relative or absolute refractoriness to histamine together with irresponsiveness to adrenaline, and also be resistant to the vaso-constriction responsible for Bier's

spots. Should the minute vessels not respond to such stimuli in the expected manner and provided any deviation in response is gross and cannot be correlated with the responses to the other stimuli, then the idea of the participation of an H-substance in the vascular reactions in these conditions will become untenable. With regard to the types of reaction under investigation in all cases the presence of some epidermal injury is evidenced by the desquamation which is associated with or follows the lesion, while

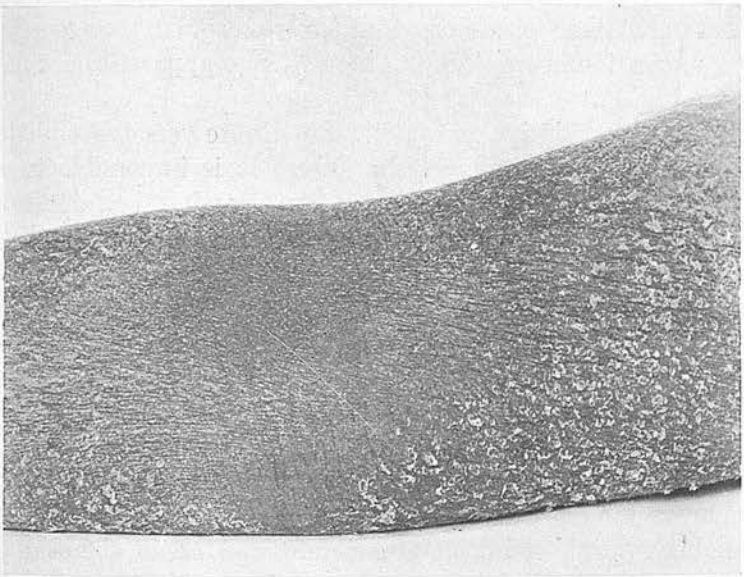

FIG. 1. EXFOLIATIVE DERMATITIS. SKIN OF ARM, SHOWING ERYTHEMA AND SCALING

microscopically vacuolation of the epidermal cells, cloudy swelling, spongiosis, imperfect keratinisation and disorganisation of the structure of the epidermis by leucocytic infiltration is present. The cause of the disease, or nature of the injury, is certain in three cases, namely, tinea corporis, ultra-violet erythema and erythema due to oil of mustard. In the case of dermatitis the cause was some irritant met with in the patient's occupation, and in the light of present day knowledge an allergic mechanism was undoubtedly involved in the production of the lesions. The cause

of the exfoliative dermatitis was uncertain, but here again allergy probably played a part. The cause of psoriasis is unknown.

EXPERIMENT 1. GENERAL EXFOLIATIVE DERMATITIS

Mrs. T. aged fifty-five. Suffering from general exfoliative dermatitis which involved the entire body surface with the exception of the nose, and the atrophic skin left by a previous varicose dermatitis. The skin was of a diffuse dull red colour, and was covered with numerous silvery scales. This condition had been present unchanged for the previous two years. On the arms there was a suggestion of speckling. There was no apparent oedema of the skin (fig. 1).

In general exfoliative dermatitis the minute vessels are dilated, hence the diffuse redness of the skin. It is impossible to say whether this vaso-dilatation is active or passive, i.e., due to an increased blood flow through actively dilated arterioles. If the latter should be the case, then the condition is analogous to the acute scarlatiniform rash.

Vascular responses

1. Congestion of the forearm with a pressure of 80 mm. Hg applied by means of a sphygmomanometer cuff produced a mottled appearance of the skin due to the development of scattered areas of a brighter red than that of the surrounding dull red or slightly purple skin. These bright red areas signified the presence of an underlying arteriolar dilation and were of the nature of a flare (see Lewis,congestion text).

2. Congestion followed by arrest of the circulation to the limb caused the appearance of Bier's white spots on the erythematous skin after an interval of three to five minutes. These spots increased in size, coalesced, and in thirty minutes a considerable area of skin on the upper and proximal part of the forearm had become dead white (fig. 2). Bier's spots were also produced on the erythematous skin of the leg.

3. Histamine puncture (1:10,000) on the chest and forearm produced a well marked triple response, including wheal forma-

tion. The local vaso-dilation was verified by histamine puncture on the forearm following preliminary congestion and arrest of the circulation to the limb, and the flare was demonstrated by the congestion test.

4. Adrenalin puncture (1:1,000) produced complete blanching of the reddened skin of the chest and forearm. Several punctures were made close together in a line to produce an area of blanching of considerable size. It was noted that a small area of vaso-

FIG. 2. EXFOLIATIVE DERMATITIS. SHOWING HOW BIER'S WHITE SPOTS OCCUR ON ERYTHEMATOUS AND DESQUAMATING SKIN

dilatation which was resistant to the blanching action of adrenalin surrounded each puncture (fig. 3). Histamine 1:3,000 applied to the adrenalin puncture gradually produced a vaso-dilatation on the blanched area which became completely reddened. Areas blanched by adrenalin but not subsequently subjected to the action of histamine remained white for a long period. The blanching due to adrenalin puncture on the forearm resisted a congesting pressure up to 50 mm. Hg.

EXPERIMENT II. PSORIASIS

Case I. A male, aged twenty-eight, had numerous large psoriasis plaques on the trunk and limbs. The condition had commenced eight years previously, and while many plaques had disappeared and reappeared the patient had never been entirely free from the disease.

Case II. A female aged sixteen, had had psoriasis plaques on the forearms and elbows for two years.

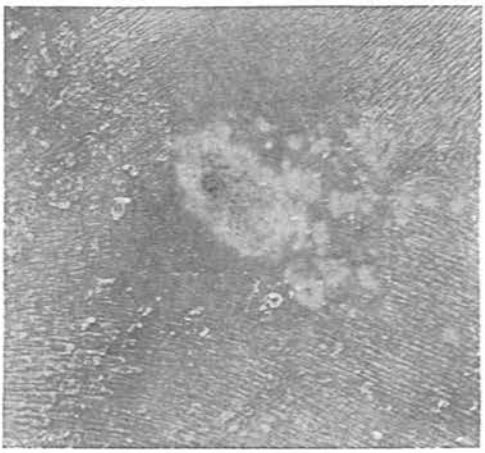

FIG. 3. EXFOLIATIVE DERMATITIS. SHOWING THE BLANCHING ACTION OF ADRENALINE (1:1000) ON THE ERYTHEMATOUS AND SCALY SKIN OF THE CHEST
Note the red reactions to injury surrounding each puncture.

Case III. Male, aged twenty-four, had numerous large psoriasis plaques on the trunk and limbs. During the preceding six years the patient had suffered from recurring exacerbations of the disease.

Case IV. A male, aged thirty, was suffering from an acute attack of psoriasis affecting the limbs. The individual lesions were numerous, small and closely set and had coalesced in places to form plaques.

In psoriasis the minute vessels are dilated, and it would seem that this dilatation is an active one. The reason for this statement is the diffuse dull red colour of the plaque or individual spot,

and the fact that this redness is clearly demarcated from the surrounding normal skin. A certain amount of oedema is also present in the papillary layer of the dermis, and is evidenced by a slight elevation of the spot, especially at the edge. The following observations were made:

1. On congesting the arm the plaques became purplish, and the area of skin immediately surrounding them was slightly pallid when compared with the bluish skin situated further away. This pallid zone, although not well marked, appeared to be analagous to the pallid zone described by Lewis, and which he found surrounding and subsequently invading the flare (Lewis, p. 202).

2. Congestion followed by circulatory arrest caused Bier's spots to appear on psoriasis plaques in 3 cases. In the fourth case Bier's spots were produced on the arm but not in the neighbourhood of the only psoriasis plaque present on the upper surface (case II).

3. In all cases histamine puncture (1:10,000 solution) produced local vaso-dilatation and whealing on the psoriasis plaques. A flare could not be demonstrated. The local action on the minute vessels was entirely comparable as regards intensity and rapidity of development to that produced on the adjacent normal skin. The reaction to histamine could be obtained on the edge of the plaque as well as on the central area.

4. In all cases adrenalin puncture (1:1,000 solution) produced complete local blanching of a psoriasis plaque. Adrenalin puncture on the normal skin in close proximity to the edge of a plaque caused an area of blanching which invaded this edge. Small areas of vaso-dilation surrounded the adrenalin punctures in all cases, and were not overcome by the adrenalin blanching.

EXPERIMENT III. PAPULO-VESICULAR DERMATITIS ON ARM

A girl, aged twenty, suffered from a confluent papular dermatitis on the back of the hands and on the forearms. The condition had been present for three months and was probably due to french polish. It was still in an active state, as evidenced by the

constant appearance of fresh vesicles on the affected area, and a tendency to spread at the edge.

1. Congestion followed by circulatory arrest caused Bier's white spots to appear on the area affected by the dermatitis

2. Histamine puncture (1:10,000) produced a triple response on the affected area, but the reaction was not so well marked as that occurring on normal skin.

3. Adrenalin puncture (1:1,000) produced an area of complete blanching on the patch of dermatitis.

Both the histamine and adrenalin punctures were laid down on areas on which fresh vesicles were scattered.

EXPERIMENT IV. TINEA CORPORIS

A patch of tinea corporis of one week's duration situated on the shoulder was examined. In tinea corporis the active dilatation of the minute vessels is evidenced by the clearly defined margin of the patch. A certain amount of papillary and epidermal oedema is also present.

Adrenalin punctures (1:1,000) were carried out on the central portion of the lesion and at the edge. In both situations full blanching was obtained. Histamine puncture (1:10,000) produced slight oedema.

EXPERIMENT V. ERYTHEMA PRODUCED BY ULTRA-VIOLET IRRADIATION

A square of skin marked out on the forearm was subjected to ultra-violet irradiation. The surrounding skin was protected from the rays, the source of which was a mercury vapour lamp. Six hours after the exposure the skin was bright red and slightly swollen, the edges of the affected area being crisply defined from the surrounding skin. In this condition the minute vessels are actively dilated, and their permeability is increased. Later on there is evidence of a slight surrounding flare.

1. Congestion of the arm followed by complete circulatory arrest caused Bier's white spots to appear on the erythematous area (fig. 4).

2. Histamine puncture (1:10,000) produced local vaso-dilation and whealing. When this local vaso-dilation was produced after the circulation to the limb had been arrested it was not invaded by Bier's spotting. On the contrary the red reaction to simple injury was obliterated by Bier's spots (fig. 4). This observation

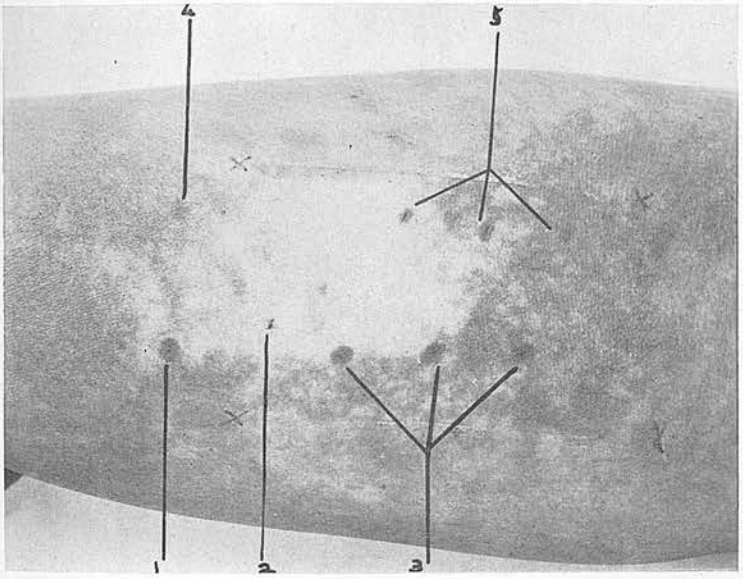

FIG. 4. ERYTHEMA DUE TO ULTRA VIOLET RADIATION

XXXX mark the four comers of the erythematous patch. The arm has been congested and the circulation arrested for twenty minutes, numerous Bier's spots of varying sizes have developed on the erythematous area. *1* = 1:3000 histamine on normal skin. *2* = simple injury. *3* = 1:3000 histamine on the erythematous patch. *4* = 1:10,000 histamine on normal skin. *5* = 1:10,000 histamine on the erythematous patch. All the punctures and the scratch were laid down after circulatory arrest had been established. Note that a large Bier's spot has blanched the red reaction to injury (*2*), but has not affected the 1:10,000 histamine puncture (*5*).

which has been repeated several times on normal skin, confirms that previously made by Rous and Gilding, who incidentally used a higher dilution of histamine in their experiments.

3. Adrenalin puncture (1:1,000) caused complete blanching of the reddened skin, but the skin in the immediate neighbourhood of the puncture showed the reaction to injury (fig. 5).

EXPERIMENT VI. ERYTHEMA PRODUCED BY OIL OF MUSTARD

A mustard plaster was applied to the skin of the forearm for twenty minutes, and a bright red erythema was produced. The reddened skin was oedematous. Similar tests to those made on the ultra-violet erythema were carried out on the oil of mustard

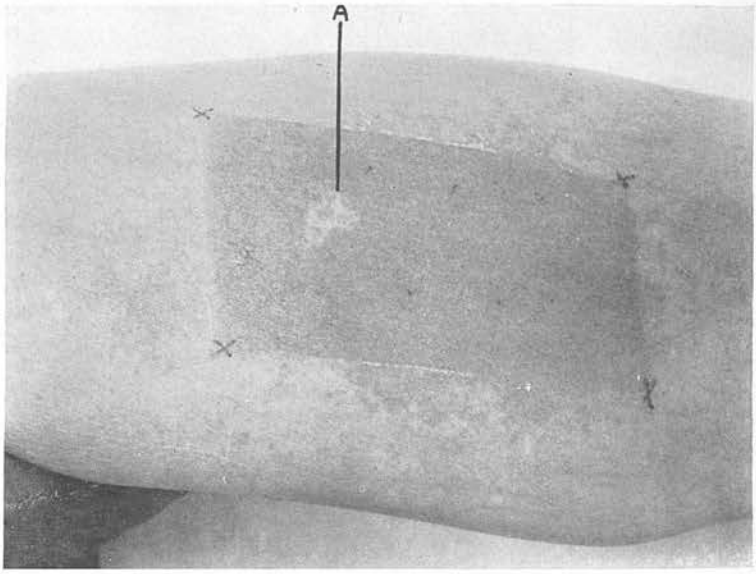

FIG. 5. ERYTHEMA DUE TO ULTRA-VIOLET RADIATION

At *A* the blanching action of adrenaline 1:1000 is shown. Note the red reaction to injury surrounding the punctures in the centre of the blanched area. The photo was taken after the reactive hyperemia following release of the circulation, which had been arrested for figure 4, had subsided. The surrounding flare is due to previous histamine punctures on the erythema and on the neighbouring skin (compare fig. 4).

erythema, and the results were identical to those obtained in the former case.

Table 1 represents the results of Experiment I to VI.

Table 2 represents the vascular responses to histamine and adrenaline in the types of erythema under consideration, which might have been expected had these erythemata been due to the action of an H-substance.

TABLE 1

Effect of histamine (1:10,000) and adrenalin (1:1,000) puncture in various types of erythema

TYPE OF ERYTHEMA	HISTAMINE	ADRENALIN
Generalised exfoliative dermatitis	Local vaso-dilatation. Oedema	Marked blanching
Psoriasis	Local vaso-dilatation. Oedema	Marked blanching
Dermatitis	Local vaso-dilatation. Oedema	Marked blanching
Tinea Corporis	Oedema	Marked blanching
Ultra-violet erythema	Local vaso-dilatation. Oedema	Marked blanching
Oil of mustard erythema	Local vaso-dilatation. Oedema	Marked blanching

TABLE 2

TYPE OF ERYTHEMA	HISTAMINE (1:10,000)	ADRENALIN (1:1,000)
General exfoliative dermatitis	No response or a very slight response	No response or slight paling
Psoriasis	No response or a very slight response	No response or slight paling
Dermatitis	No response or a very slight response	No response or slight paling
Tinea corporis	No response or a very slight response	No response or slight paling
Ultra-violet erythema	No response or a very slight response	No response or slight paling
Oil of mustard erythema	No response or a very slight response	No response or slight paling

DISCUSSION

The exact condition of the minute vessels in all the skin lesions investigated is not known with absolute certainty. In every case they are dilated, and presuming that a crisp edge to an erythema indicates an active state of vasodilatation, then in psoriasis, ultra-violet erythema and tinea corporis, he existing vaso-dilatation is an active one. Assuming that all the erythemata are due to liberation of H-substance, it follows that the concentration of H-substance necessary to produce an arteriolar dilatation accom-

panied by passive dilatation of the minute vessels would also cause an active dilatation of minute vessels through its direct action on them. A flare which is unaccompanied by an active vaso-dilatation of minute vessels can hardly be attributed to the action of an H-substance. On the other hand, it is conceivable for an H-substance to act in such a concentration that it produces its local effect on the vessels without exciting any vaso-dilator reflex. If, therefore, the present types of erythemata are assumed to be due to H-substance activities it follows that the dilatation of the minute vessels must be due in part, if not wholly, to the direct action of H-substance on the walls of these vessels.

As has already been mentioned, vessels which are already under the influence of histamine, or those which have responded to injury and are presumed to be under the influence of H-substance, show a modified response to histamine and adrenalin puncture. Furthermore the local vaso-dilatation produced by histamine is not invaded by Bier's spots (white), whereas the corresponding vaso-dilatation associated with skin injury is obliterated by these spots. This discrepancy may be due to the concentration of histamine employed, although in Rous and Gilding's experiments the visible reaction was the same in both types of vaso-dilatation.

The results obtained in the experiments described above have been interpreted on the basis of the foregoing facts and assumptions.

The reaction of the already dilated minute vessels to histamine 1:10,000 in the various types of erythema investigated is interesting, but yields no information concerning the stimulus responsible for the existing vascular reaction. The response to histamine merely indicates that the existing state of vaso-dilatation has not been complete, and that the vessels are therefore capable of further dilatation if a stronger stimulus is applied to them. Under such circumstances it is to be expected that the permeability of these partially dilated vessels will be affected by histamine, and that refractoriness, if present, will be incomplete. This is exactly what has been observed, for in all cases whealing, of an apparent maximum degree for the strength of stimulus applied, has been obtained with histamine. Even in view of the

incomplete nature of the vascular response of the erythemata in question this seemingly complete absence of refractoriness to histamine is somewhat inconsistent with the hypothesis that the substance acting on the vessels and responsible for their incomplete state of dilation is an H-substance.

The reactions of the vessels in these erythemata to 1:1000 adrenaline provide almost conclusive evidence that the vaso-dilatation can not have been produced by an H-substance. In each case adrenaline puncture produced well marked blanching of the reddened skin. This lack of irresponsiveness to adrenaline was in accordance with the increased vaso-dilatation produced by histamine, and with the absence of refractoriness. Its occurrence suggested a passive rather than an active dilatation of the vessels responsible for the skin colour, in other words of the minute vessels. This being so, the whole vascular reaction provoked by the stimulus responsible for the particular type of erythema could be explained as being of the nature of a flare, and thus related to the type of reaction present in the scarlatiniform rash.

Since the presence of a flare has been taken as an indication that a stronger stimulus than is required to act on the minute vessels is in operation, it is difficult to correlate the combination of a well marked flare with passive dilatation of the minute vessels and at the same time to attribute the entire reaction to an H-substance. Furthermore the minute vessels round the adrenaline puncture dilate in response to the stimulus of the injury—as pointed out by Lewis when dealing with irresponsivenexss to vaso-constrictor substances. *The redness produced by this vaso-dilatation is not more marked than that of the lesion on which the puncture has been laid down, yet the stimulus which has produced it has been sufficient to overcome the vaso-constricting action of adrenaline. If it is admitted that the vaso-dilatation produced by the injury is directly due to the action of a liberated H-substance, then a similar substance can hardly be responsible for the vaso-dilatation present on the already erythematous skin, since the latter is overcome by adrenaline.* To correlate these two observations it would be necessary to postulate a much lower concentration of H-substance

in the diseased skin than had been liberated at the point of injury. Such an hypothesis would be inconsistent with that which suggests that the vascular reaction of the reddened skin comprised for the most part a flare, since the intensity of the latter reaction as judged by colour is at least as great as that due to slight injury. *If a liberated H-substance is responsible for the vascular response to injury, then the facts observed do not warrant the conclusion that the vascular reactions occurring in the various types of reddened kind under consideration are due to a similar substance and vice versa.*

The further discrepancy namely that the influence which produces the constriction of small vessels in Bier's spots also constricts the dilated small vessels in areas reddened by disease, by injury and by physical and chemical agents, while not invading the areas reddened by histamine, is additional evidence against the intervention of the same H-substances in the vascular responses of the skin to all types of injury, including that produced by disease.

A comparison of the vaso-constricting action of adrenaline and Bier's spots on the local redness due to trauma shows that the latter causes complete disappearance of this redness while the former does not. From this it may be argued that should the vaso-constriction which occurs in Bier's spots be due to the action of a vaso-constrictor substance formed in the tissue spaces during the period of circulatory arrest it must be more potent than 1:1000 adrenaline.

SUMMARY AND CONCLUSIONS

When the reactions of the dilated minute skin vessels in (*a*) exfoliative dermatitis, ultra-violet erythema, oil of mustard erythema, psoriasis, dermatitis venenata, and tinea corporis (ring-worm) (*b*) trauma, and (*c*) histamine local vaso-dilation, to adrenalin puncture and to Bier's spots are compared it is found that each group reacts differently. In group 1 adrenalin overcomes the existing local vaso-dilatation, while it does not influence that present in groups 2 or 3. Bier's spots overcome the vaso-dilatation in groups 1 and 2, but have no effect on group 3. These facts do not support the hypothesis that the vaso-dilatation

which is common in each group has been produced by a common chemical substance liberated in the tissues. There is, however, no evidence to show that the vaso-dilatation in group 1 has not been brought about by the liberation of some chemical substance in the same way as has been shown by Lewis to occur in group 2.

Nevertheless it cannot be concluded on the basis of the present observations that any chemical substance which may be liberated in the tissue spaces and be responsible for the vascular reactions included in group 1 is a histamine-like substance or histamine itself.

The observations that the local vaso-dilatation to mechanical injury is overcome by Bier's spots (Rous and Gilding) and is resistent to adrenalin blanching (Lewis) have been confirmed.

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OBSERVATIONS ON THE ÆTIOLOGY OF ERYTHEMA EXUDATIVUM MULTIFORME

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OBSERVATIONS ON THE ÆTIOLOGY OF ERYTHEMA EXUDATIVUM MULTIFORME.

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THERE are two views regarding the ætiology of erythema exudativum multiforme. The first recognizes in this condition a true disease entity, the cause of which, although as yet undiscovered, is provisionally held to be the same in all cases. In contra-distinction to this morbid entity are ranged a whole series of multiform erythemata occurring during the course of intoxications or infections of known origin, a few of which bear a close resemblance to true erythema multiforme. The majority, however, only merit the description "multiform" in virtue of the varied appearance of the erythematous eruption, which, on close inspection, has neither the clinical appearance or course of the true disease. This type of exanthem is termed "secondary" erythema multiforme. It is, of course, admitted by the adherents of this classification that true erythema multiforme may supervene as an intercurrent and independent condition during the course of other diseases.

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The second view regards erythema multiforme as a cutaneous manifestation of a large variety, as yet unexhausted, of intoxications and infections. While admitting that in many cases the intoxication or infection is unknown, it is held that this group, provisionally classed as idiopathic, will ultimately disappear, and that it will be possible to class all cases of erythema multiforme as secondary cutaneous reactions of multiple ætiology.

The spontaneous, recurring, seasonal variety is the true disease according to the first view, and the majority of such cases are classed as idiopathic according to the second. Both views recognize the occurrence of multiform erythemata during the course of other diseases, but the first does not include such eruptions in the category erythema exudativum multiforme (1).

Investigations into the ætiology of the true or idiopathic form have so far yielded no information as to its nature. A streptococcal origin has been suggested, but hitherto blood-culture and culture of the lesions themselves have been completely negative. Focal sepsis, particularly that due to streptococcal infection, is held by many to be the cause, principally in view of the results of the elimination of such possible infected foci, and of the finding of streptococci in the teeth and tonsils. In addition, positive skin-reactions to streptococcal products have been obtained in a few cases (2). In this connection such data as are available are unconvincing. Without proper normal controls and observation of a large number of cases cuti-reactions cannot be relied on, and the uncertainty of recurrences in any one case renders deductions as to the success of the elimination of areas of focal sepsis in the cure of the disease, or in the prevention of recurrences, of little value.

The bacteriological researches of Parker and Hudson (3), and of Levaditi, Nicolau and Poincloux (4), in which they isolated a streptobacillus from the blood and skin-lesions in patients suffering from fever, joint pains and an erythematous eruption, throw no light on the problem, since the skin condition in their cases was not true erythema multiforme.

Recently Ramel (1, 5) has put forward the view that true erythema multiforme is a cutaneous tuberculous manifestation due to a scanty tubercular bacillæmia in an otherwise healthy subject.

His attention was first directed to this possibility on account of the similarity of some cutaneous tuberculin reactions to erythema multiforme, the coincidence in one case of papulo-necrotic tuberculides and

erythema multiforme, and the resemblance between severe erythema multiforme and acute lupus erythematosus—a condition which he regards as being of tuberculous origin.

In support of this theory Ramel claims to have demonstrated the presence of tubercle bacilli in the blood of ten patients suffering from erythema multiforme, in whom there was no other clinical sign of tuberculosis. These results were obtained by the method of successive guinea-pig inoculation. Citrated whole blood or the centrifuged residue of lysed blood was injected subcutaneously into guinea-pigs. These were left for two to six months, and during that time thrived normally. A post-mortem examination at the end of that time revealed slight adenitis of the ilio-lumbar and mesenteric glands, enlargement of the liver with some fatty change, splenic enlargement, mottling and some fibrosis of the lungs. These changes were actually noted in animals killed as early as the twentieth day. The affected organs were removed, macerated, and injected into a second series of guinea-pigs. Again the animals thrived, and at the post-mortem examination the same changes were found as had been observed in the first animals. Inoculation of the organs was again repeated, and at the second to fourth passage signs of tuberculosis were noted, starting from the point of inoculation, and acid-fast bacilli were found in caseous lymph-glands. Thereafter subsequent passage produced a rapidly fatal tuberculosis in the guinea-pig. At this stage tubercle bacilli of the human type were cultivated from animals originally associated with blood inoculation from two cases of erythema multiforme. In one case tissue round the site of inoculation was taken from a guinea-pig twenty days after the injection of blood. This tissue was re-inoculated, and tuberculosis was produced at the third passage. The guinea-pigs in Ramel's first series of six cases remained tuberculin-negative until a definite clinical and bacteriologically proved tuberculosis had been established. There is no mention made of testing the animals prior to inoculation, and the tuberculin test was apparently not used in the second series of four cases. With regard to the tuberculin test in the patients examined, it is stated that in one case which was associated with a series of animals which developed tuberculosis the test was positive, and in another similar case negative. No control experiments on the effect of successive inoculation of a series of guinea-pigs with the organs of other guinea-pigs, or a repetition of the whole experiment using healthy individuals not suffering from erythema multiforme, were performed. The

blood of a case of erythema nodosum was, however, examined by this method, and tuberculosis was produced in a guinea-pig at the second passage.

A similar investigation in a series of ten cases of erythema multiforme and six cases of other types of skin-disease as controls has been carried out by the authors.

Before attempting to demonstrate the presence of tubercle bacilli or a related virus in the blood-stream of cases of erythema multiforme, it was recognized that rigid control was necessary at all stages of the experiment. Before it could be concluded that an organism, isolated after repeated animal passage, was derived from the original inoculum, the possibility of latent infection of the animal used had to be excluded. In the present series the guinea-pigs used were subjected to an intradermal test with a standard sample of old tuberculin, diluted a short time before use. The dilutions used (1 in 100 or 1 in 500) were such that latent infection would at once have been revealed. In the absence of this precaution, the method of inoculating the pooled tissue emulsion from two guinea-pigs into two further animals at each stage, was one which would distribute the organism from one infected animal among all the remaining animals of the series.

Further control of the animal stock employed and of their environment during the course of the prolonged experiment was afforded by a parallel series of passage experiments following inoculation with blood from skin cases other than erythema multiforme. In addition, this series was used to reveal any fallacy arising from the technique of inoculation and treatment of the tissues. The hypothesis that erythema multiforme was of tuberculous origin would at once have been invalidated if positive results in the test series had been paralleled by similar findings in the control series of cases in which the presence of that disease could be definitely excluded.

B. tuberculosis might exist in the blood in numbers too small to produce progressive lesions in the experimental animal. It might also be present in an attenuated form capable of producing only the atypical lesions described following inoculation with the so-called filtrable form of the organism ([6, 7]).

In these cases complete resolution of the lesions and disappearance of the bacilli might occur if passage inoculation were too long delayed. For this reason the time which was allowed to elapse between inoculation and

the killing of the animal was varied so as to secure, at least in some cases, the optimal enrichment of any infective material present. By passage through four or five sets of animals sufficient opportunity was given for increase of the number, or exaltation of the virulence of any organisms present in the original inoculum from the patient.

Technique.

Five to 10 c.c. of blood withdrawn from the median basilic vein were thoroughly mixed with sodium fluoride to prevent clotting. Animal inoculation was carried out in all cases within three hours of collection of the blood-sample.

Lysate.—The blood was mixed with the minimum quantity of sterile distilled water necessary to secure complete lysis of the red corpuscles. The mixture was rapidly centrifuged at 3500 r.p.m. for one and a half hours, when the supernatant fluid was pipetted off, leaving a volume of about 5 c.c., which was divided into two equal parts for inoculation.

Whole blood.—The blood in some cases was inoculated directly into the animal without preliminary lysis.

Treatment of animal tissues.—At autopsy portions of lung, liver, spleen and kidney were removed. In addition any lesion at the site of previous inoculation, usually seen as a small fibrous nodule, and any enlarged lymphatic glands in the left inguinal region or elsewhere were also taken.

The tissues were ground in a sterile mortar with sand and emulsified in 5–10 c.c. of sterile 0·85% salt solution. The sand and tissue *débris* were allowed to sediment by gravity, and the turbid, blood-stained supernatant fluid taken for inoculation into further animals.

Inoculation.—Two guinea-pigs were inoculated subcutaneously into the left groin on each occasion either with the centrifuged lysate, whole blood or tissue extracts. The extracts made from the tissues obtained post-mortem from both animals were mixed, and the combined extract re-inoculated into two animals. Where the volume of fluid was large the needle was carried up into the subcutaneous tissue of the left flank to avoid distension of the tissues, with the possibility of necrosis of the skin or underlying muscles.

Tuberculin testing of guinea-pigs.—All animals were tested prior to inoculation and just before they were killed. A sample of Koch's old tuberculin was used, which had recently been shown by comparative tests

on tuberculized guinea-pigs to be of full potency when compared with a standard sample supplied by the National Institute of Medical Research, London. The tuberculin used had produced marked reactions in a dilution of 1 : 4000.

A dilution of 1 : 500 in 0·5% carbol-saline was made up shortly before use. The test was an intradermal one, 0·2 c.c. of dilute tuberculin being injected into the shaved skin of the animal's belly. The reaction was observed in twenty-four and forty-eight hours. No animals were found to give any trace of reaction. During the latter part of the work a tuberculin dilution of 1 : 100 was used to make the test still more searching. Again the results were uniformly negative. The stock animals of this laboratory have been free from spontaneous tuberculosis for a number of years.

Summary of Cases and Results of Guinea-pig Inoculation.

Cases of Erythema Multiforme.

Case 1.—A typical erythema multiforme eruption appeared on the forearms, and backs and palms of both hands, three weeks after a burn on the left elbow fold. This was the patient's first attack of erythema multiforme. Physical examination, including X-ray of the lungs, revealed no active focus of tuberculous infection. The tuberculin test was negative to human and bovine tuberculin. Blood was obtained for examination and guinea-pig inoculation on the second day of the eruption.

Blood-culture : Negative.

Summary of guinea-pig inoculations :

Length of experiment	71 weeks
Number of passages	3
Length of passages	15–26 weeks
Number of guinea-pigs used . . .	8
,, deaths	1

Case 2.—Male, aged 39 years, suffering from erythemato-papular and bullous eruption of erythema multiforme affecting the nuchal region, backs of arms and hands, legs and feet. Commenced suddenly, and was associated with slight shivering and a temperature of 101° F. for eight hours. The lesions continued to appear for the next few days, and the

eruption gradually subsided during the following three weeks. The patient has had three previous attacks, each one occurring in August. His general health was good, and physical and radiological examination revealed no evidence of active tuberculosis. The tuberculin reaction was negative to human and bovine tuberculin. Blood was obtained for examination and guinea-pig inoculation on the second day of the eruption.

Blood-culture : Negative.

Summary of guinea-pig inoculations :

Length of experiment	69 weeks
Number of passages	3
Length of passages	13–26 weeks
Number of guinea-pigs	10
,, deaths	5

Case 3.—Male, aged 18 years. The patient complained of an attack of erythema affecting the backs of both hands. During the past seven years the patient had had similar attacks twice yearly, lasting about fourteen days, one of which had always occurred in August. Various treatments, including streptococcal and staphylococcal vaccines and autohæmotherapy, had been used. Physical and radiological examination revealed no evidence of active tuberculosis. The tuberculin reactions to human and bovine types were negative. Blood for examination and guinea-pig inoculation was obtained on the third day of the eruption.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	65 weeks
Number of passages	3
Length of passages	12–23 weeks
Number of guinea-pigs	10
,, deaths	3

Case 4.—Male, aged 35 years. A papulo-erythematous eruption of erythema multiforme appeared suddenly on the backs of the hands and nuchal region. The patient had had no previous attacks. The eruption was unaccompanied by any general symptoms. Physical and radiological examination did not reveal any trace of an active tuberculous focus.

The eruption lasted for four weeks. The tuberculin reaction was negative, and 20% old tuberculin ointment rubbed into the lesions produced no reaction. Blood was obtained for culture and guinea-pig inoculation on the third day of the eruption.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	70 weeks
Number of passages	3
Length of passages	7–29 weeks
Number of guinea-pigs	10
Number of deaths	1

Case 5.—Female, aged 29 years. Patient complained of an erythema multiforme eruption on the backs of both hands. During the next few days the lesions continued to appear, affecting the forearms and lips. She had had numerous similar attacks during the previous nine years. Apart from the eruption the patient was perfectly well, and physical and radiological examination revealed no sign of active tuberculosis. The tuberculin reactions were negative, and 20% old tuberculin ointment rubbed into the lesions produced no reaction. Blood was obtained for culture and guinea-pig inoculation on the second day of the eruption.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	60 weeks
Number of passages	2
Length of passages	16–22 weeks
Number of guinea-pigs	8
„ deaths	2

Case 6.—Male, aged 20 years. The patient complained of an erythema multiforme eruption on the backs of both hands and arms. This was his third attack. Physical and radiological examination revealed no sign of active tuberculosis. The tuberculin reactions were negative, and inunction of the lesions with a 20% old tuberculin ointment produced no reaction. The eruption faded in fourteen days. On the second day blood was obtained for culture and guinea-pig inoculation.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	73 weeks
Number of passages	3
Length of passages	16–23 weeks
Number of guinea-pigs	8
,, deaths	0

Case 7.—Male, aged 20 years. The patient was suffering from erythema multiforme affecting the lips, buccal mucous membrane and backs of hands. There had been very frequent attacks during the previous three years, which removal of tonsils had failed to abate. Apart from the buccal lesions and slight itching the patient was in good health, and physical and radiological examination did not reveal any sign of tuberculosis. The tuberculin reaction was negative, and inunction into the skin-lesions of 20% old tuberculin ointment produced no reaction. The eruption lasted for three and a half weeks, and blood was obtained for culture and guinea-pig inoculation on the fifth day.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	68 weeks
Number of passages	3
Length of passages	16–24 weeks
Number of guinea-pigs	8
,, deaths	0

Case 8.—Male, aged 11 years. The patient was suffering from erythema multiforme affecting the mouth and lips, the arms, legs, hands and feet. The eruption was not accompanied by any general symptoms. Fresh lesions appeared for a day or two and the eruption lasted five weeks. The patient had had four similar attacks during the previous ten months. He had had a tuberculous arthritis of the knee in infancy, but this was pronounced cured at the age of four. There was a resulting ankylosis of the knee-joint. Physical and radiological examination showed no sign of active tuberculosis. No reactions occurred with human or bovine tuberculin, nor with 20% old tuberculin rubbed into the skin-lesions Blood was withdrawn for culture and guinea-pig inoculation on the fifth day, when fresh lesions were still appearing.

Blood-culture : Negative.

Results of guinea-pig inoulation :

Length of experiment	64 weeks
Number of passages	2
Length of passages	24 weeks
Number of guinea-pigs	6
„ deaths	1

Case 9.—Female, aged 34 years. The patient was suffering from erythema multiforme affecting the lips, inside of the mouth, and the backs of the hands and arms. Clinical and radiological examination showed no signs of any active tuberculosis. The tuberculin tests were negative. During the previous three years the patient had had numerous attacks of erythema multiforme. Removal of all the teeth had failed to stop these recurrences. The present eruption lasted for about six weeks. On the fifth day of the eruption blood was withdrawn for culture and guinea-pig inoculation.

Blood-culture : Negative.

Results of guinea-pig inoulation :

Length of experiment	60 weeks
Number of passages	2
Average length of passages	24 weeks
Total number of guinea-pigs	6
Number of deaths	0

Case 10.—Female, aged 17 years. The patient was complaining of erythema multiforme affecting the backs of the hands, arms, feet and legs, and the lips. The attack lasted for four weeks, and was the sixth recurrence during the previous three years. There was no evidence of active tuberculosis, either clinically or radiologically. The tuberculin tests were negative, and 20% old tuberculin ointment rubbed into the lesions produced no reaction. Blood was obtained on the fifth day of the eruption.

Blood-culture : Negative.

Results of guinea-pig inoculation :

Length of experiment	53 weeks
Number of passages	2
Length of passages	13–24 weeks
Total number of guinea-pigs	6
Number of deaths	1

Summary.

In ten cases of erythema exudativum multiforme blood-cultures carried out during the period of active spread of the disease were entirely negative.

Inoculation of tuberculin-negative guinea-pigs with blood from these cases, followed by successive inoculation of extracts of the viscera of these animals into other tuberculin-negative animals, according to the method of Ramel, did not give rise to any tuberculous lesions in the later animals. The early non-specific lesions described by Ramel were not observed. No positive tuberculin reactions were found to develop. The animals which survived thrived normally, and maintained or increased their weight during the course of the experiment.

The experiments may be summarized as follows :

Average total length of experiments . .	63·5 weeks
,, ,, ,, first inoculation .	12·0 ,,
,, ,, ,, passages . . .	19·9 ,,
Total number of guinea-pigs used . .	80
Average number of guinea-pigs per case .	8
Total mortality	14
Percentage mortality	17·5%

In the fourteen guinea-pigs which died during the course of the experiments death was due either to enteritis, generalized septic peritonitis, pasteurellosis, or *salmonella* infection. Details of two passage experiments are given in full in Table I (p. 12).

To control the foregoing experimental results a similar investigation was carried out on the blood of six patients suffering from skin-diseases other than erythema exudativum multiforme. These cases included palmar and plantar hyperkeratosis, dermatitis, chronic leg ulcers, psoriasis, acne vulgaris, impetigo contagiosa.

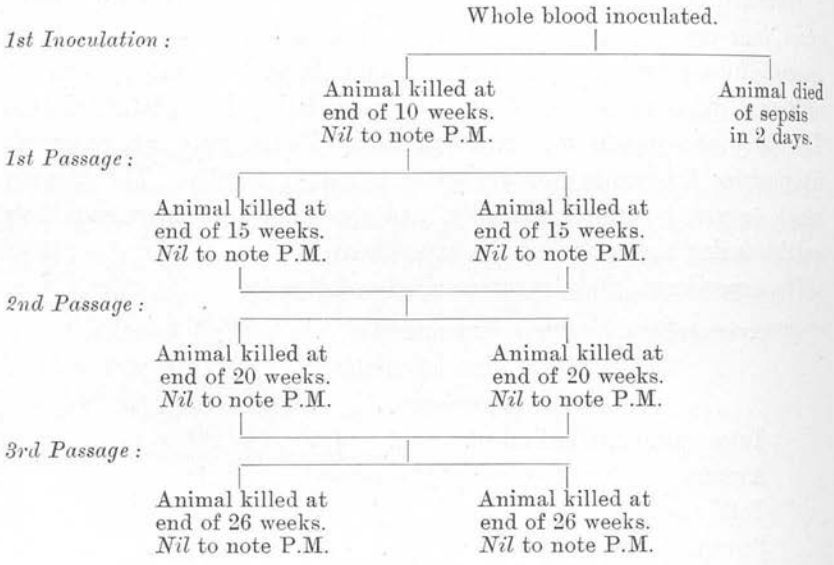
In each case blood-culture was negative.

The results of guinea-pig inoculation were as follows :

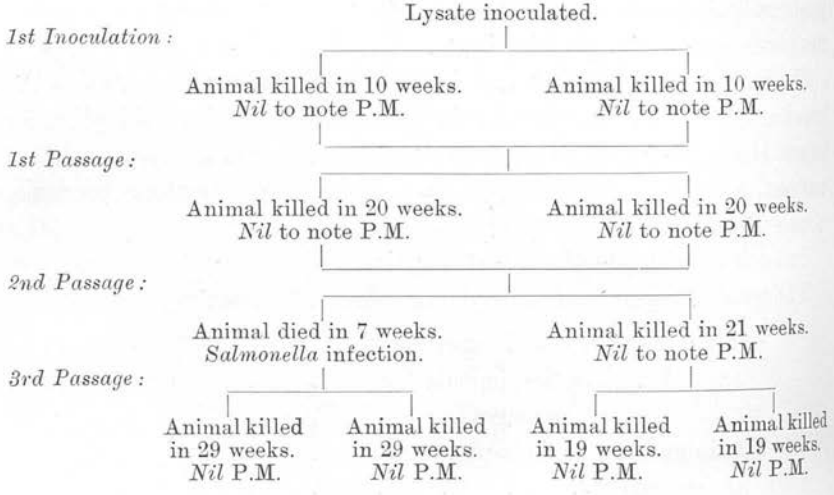
Average total length of experiment . .	56·5 weeks
,, length of first inoculation . .	11·0 ,,
,, ,, passages	16·6 ,,
Total number of guinea-pigs used . .	46
,, mortality	16
Percentage mortality	34·8%

TABLE I.—*To Illustrate the Method of Successive Guinea-pig Inoculation*

CASE 1: ERYTHEMA MULTIFORME.



CASE 4: ERYTHEMA MULTIFORME.



All the animals were tuberculin-negative before inoculation, and none developed a positive tuberculin reaction during the course of the experiment. Post-mortem examination did not reveal any local or visceral tuberculosis. The animals which survived and were ultimately killed maintained or increased their weight during the period of the primary inoculation or subsequent passages. In the sixteen animals which succumbed during the course of the experiments death was due to acute non-tubercular infections of the lungs, bowel or peritoneum.

Discussion.

The investigations carried out by the authors have entirely failed to confirm the results obtained by Ramel, on which is based his theory of the tuberculous nature of all cases of true or idiopathic erythema exudativum multiforme. The cases investigated in the present research correspond exactly in all their clinical details to those of Ramel's series, and the technique which he employed has not been varied. Indeed, with regard to technique, there is little possibility of any great divergence, since the object is to concentrate any bacilli which may be present in the blood of the patient into as small a bulk as possible—a procedure which is entirely dependent on adequate centrifugalization. Neither does the procedure of injecting emulsions of organs from one animal into another present any difficulty, or require any elaborate or highly skilled technique in its performance. The duration of the experiments is similar to that of the three published in detail by Ramel, in which the passages averaged 13·5 weeks, and in which definitely tuberculous lesions containing acid-fast bacilli were found to occur in the second passage animal 36 weeks, on an average, from the inoculation of blood into the first animal. The present experiments cannot, therefore, have failed on account of the duration of the passage periods varying appreciably from those of Ramel. In no case did a tuberculin reaction develop in the present series of animals. Ramel, who does not mention that his animals were tested prior to inoculation, found a positive tuberculin reaction only in those inoculated animals which showed a definite tuberculosis post mortem. The reaction was absent in the early animals, which only showed vague indeterminate lesions. Ramel did not carry out a series of control observations with the blood of normal individuals or perform successive inoculations of guinea-pigs' viscera. This is a necessary procedure when

the question of raising the virulence of an organism is concerned, since an apparently healthy stock of animals may actually be infected with an attenuated form of the virus under investigation. This is doubly important in the case of tuberculosis—a disease to which laboratory animals are to some extent prone. It is to be noted that in Ramel's animals there was nothing to note beyond simple inflammation at the point of inoculation in the first animals inoculated with the patient's blood. Furthermore, a chronic inflammatory process spreading from the point of inoculation was only noted in the later animals, which had been injected with the viscera of those previously inoculated.

The experimental results obtained by Ramel can only be correlated with those of the present investigation by discarding the hypothesis that erythema exudativum multiforme is a true morbid entity with but one single cause, and reverting to the conception of erythema multiforme as a cutaneous reaction capable of being produced by a variety of agents. From this standpoint a tuberculous type of erythema multiforme which has not been encountered in the present series of cases could be admitted, and Ramel's work would, to this extent, have depleted the ranks of the idiopathic variety.

It is to be noted that in the present experiments no organisms of any description were found in the blood during the acute spreading stage of the disease.

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EXPERIMENTAL OBSERVATIONS ON DERMATITIS DUE TO DYED FUR.

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IN the winter of 1922–23 it became apparent that the incidence of cases of dermatitis due to the wearing of dyed fur had suddenly increased. Before that period dyed fur had been recognised as a cause of dermatitis, both among those engaged in the preparation of such articles and among those wearing them, but, nevertheless, examples of this type of eruption were rarely seen. The increase noted in 1922–23 was maintained during the winter of 1924, and during these years several papers dealing with the subject were published. In 1924 the Ministry of Health conducted an inquiry into the occurrence of dermatitis attributed to the wearing of fur collars. The main conclusions arrived at in the report of the Ministry [1] (which were based on facts obtained during the inquiry and on papers dealing with the subject in the current medical journals) were as follows: Personal idiosyncrasy on the part of the patient was responsible to some extent for the appearance of the dermatitis, but this susceptibility was specific for a certain class of fur, and did not explain the sudden and unprecedented incidence of fur dermatitis cases. The dermatitis was apparently produced by products of the incomplete oxidation of para- and meta-phenylenediamine. In some instances the mordant used was also thought to be partly responsible. The part played by personal idiosyncrasy was obvious when the proportion of sufferers from fur dermatitis to wearers of dyed furs was considered. The facts recorded in the inquiry did not, however, provide any conclusive evidence as to the nature of the

* In receipt of a part-time grant from the Medical Research Council.

actual constituent in the fur which was responsible for the dermatitis, and any statements regarding this important point were merely tentative. During the process of dyeing, a fur may be treated with various chemicals, including the actual dye itself, and any one of these is theoretically a potential skin irritant. The fact that a particular or even chemically unidentifiable oxidation product of an aniline dye can be isolated from a fur which is known to have been treated with the mother substance of the isolated compound is no proof that such a compound is the cause of the dermatitis which the fur has produced. Direct proof of the specific irritant properties of a substance on the human skin can only be obtained from biological experiments with the substance in question. A more precise knowledge of the nature of the specific susceptibility of the skin towards the irritant may also be obtained if such methods are employed in the investigation of the problem.

Cases of dermatitis due to dyed fur, although less numerous than in the years 1922–24, are still frequently met with. Table I. shows the total number of such cases and their percentage of the total number of cases of dermatitis in females over 15 years of age who have been treated in the skin department of the Edinburgh Royal Infirmary during the decennium 1920–30 :—

TABLE I.

A = Total number of cases of dermatitis in females over 15.
B = Number of cases due to fur.
C = Percentage of total due to fur.

Year.	A.	B.	C.	Year.	A.	B.	C.
1920	174	1	0·57	1926	238	16	6·72
1921	185	1	0·54	1927	168	7	4·18
1922	135	1	0·73	1928	252	13	5·15
1923	194	22	11·34	1929	362	23	6·35
1924	233	37	15·88	1930	385	13	3·37
1925	201	17	8·45				

For obvious reasons, the complaint is almost exclusively met with in females, so that figures dealing only with dermatitis in females have been given for the total annual number of dermatitis cases in the Table. As no cases of fur dermatitis have been met with in young children, the numbers given for all classes of dermatitis have been limited to cases

over 15 years of age. The number of cases of fur dermatitis given for 1920–22 may be slightly low, since attention was not concentrated on the possibility of fur being the cause of dermatitis until the condition became prevalent in 1923. Nevertheless, the abrupt rise in 1923–24 is undoubtedly real, for the numbers were then much higher than in the succeeding years, during which the condition was well recognised.

Since the incidence of this type of eruption is still considerable, an investigation into its causation and nature has been undertaken. Furthermore, as the problem involved was a concise one, dealing with a type of dermatitis due to an irritant which could presumably be definitely ascertained, it was thought that any observations which might be made during the course of the investigation would have some bearing on the related problems presented by other types of dermatitis, either due to external or internal causes.

Investigation.

The irritant properties of two lambskin furs, one dyed brown and the other grey, which were known to have caused dermatitis, were examined. The experiments carried out with the brown fur were of a preliminary nature to test out the methods which it was proposed to use throughout the investigation, while those performed with the grey fur were more detailed, and were pursued further.

The main issues of the problem may be stated as follows :—

(1) Is the skin irritation produced by a dyed fur due to the fur itself or to the chemicals used in the dyeing process ?

(2) Are any special conditions, such as moisture or a greasy skin, necessary before a dyed fur can produce skin irritation, and, if so, will apparently normal skins react to certain dyed furs provided such favourable conditions are present ?

(3) Can a skin, which has previously tolerated a given fur, subsequently develop an eruption when again brought into contact with this fur ?

(4) What substance in the fur is responsible for the production of dermatitis ?

(5) What concentration of this substance is required in order to produce dermatitis ?

(6) Does the action of the irritant substance, when this is present in the concentration required to produce dermatitis, differ on normal skins and on the skin of subjects who have suffered from dermatitis due to the particular irritant in question ? In other words, is the irritation which is produced by the substance entirely due to a constant specific pharmacological action depending on and proportional to its concentration, or must the skin possess or develop the property of reacting to the application of the substance with a special type of reaction before the latter can produce any harmful effects ?

(7) If such a qualitative difference does exist between normal skins and those which are irritated by compounds contained in dyed fur, what is the degree of this difference ?

(8) Can the irritant properties exhibited by a given dyed fur be removed by appropriate chemical or physical manipulation ?

The method used throughout in the investigation of these questions was that of the contact eczema test.

This test was introduced by Jadassohn and Bloch to detect the external irritant in cases of dermatitis, and since its introduction it has been extensively used on the continent as a routine practice in the investigation of such cases, and in special investigations on the nature of dermatitis. The method is simple, and consists in the application of the suspected irritant in low concentration, either in solution, paste, or ointment form, to a small area of the skin of the back. Solutions are applied as soaks on small linen squares. The test application is covered with linen or oiled silk and kept in position with adhesive plaster. The application is removed at the end of 24 hours, and the skin examined. If the test is positive, the skin which has been in contact with the test substance is red and slightly œdematous, and the surface is studded with minute vesicles. It presents the appearance of a localised area of dermatitis. The area in direct contact with the adhesive plaster is frequently red and the hair follicles prominent, but this quickly subsides, although in some cases the skin is actually sensitive to the ingredients of the plaster, and the redness persists. Sometimes a positive reaction is not evident when the tests are removed and the skin examined at the end of 24 hours, but develops during the succeeding 24 hours. It is necessary, therefore, to verify all negative reactions 24 hours after removal of the test applications, in case a late positive reaction develops. A positive result is a direct proof that the substance applied is capable of producing a dermatitis in the concentration employed, and the degree of sensitivity of the skin towards the substance can be ascertained by varying the concentration of the latter.

While such tests are of immense value in elucidating the cause of individual cases of dermatitis, they have

also been used to demonstrate the relative susceptibility of the skin of patients suffering from dermatitis to react to several types of irritants, as compared with the skin of normal individuals. Thus Jaeger [2] found that 50 per cent. of cases of dermatitis gave an abnormal inflammatory reaction to certain volatile oils, while only 5 per cent. of normal individuals reacted. Using similar substances, Bloch [3] found that 35 per cent. of the dermatitis cases reacted, and only 5 per cent. of normal individuals gave positive reactions. Evening [4] obtained positive results in 36 per cent. of dermatitis cases, and 2·2 per cent. of normal individuals, and Schürch [5] obtained results of a similar order.

BROWN LAMBSKIN DYED WITH PARAPHENYLENEDIAMINE.

The first fur to be investigated, using this method, was a brown lambskin which had been dyed with paraphenylenediamine. Three female patients, who were known to have suffered from dermatitis of the

TABLE II.

Results of contact tests with paraphenylenediamine-dyed brown lambskin fur applied for 24 hours in three patients who had suffered from dermatitis due to dyed fur collars.

M.D.W. = Moistened with distilled water.
M.S. = Moistened with saline.

Case.	Patient's own fur.			Dyed brown lambskin.			Control undyed lambskin.		
	Dry.	M.D.W.	M.S.	Dry.	M.D.W.	M.S.	Dry.	M.D.W.	M.S.
1	+ + +	+ + +	+ + +	−	+ + +	+ + +	−	−	−
2	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	−	−	−
3	+	+ + +	+ + +	+	+ + +	+ + +	−	−	−

+ = redness persisting for 24 hours.
+ + = redness and œdema.
+ + + = redness, œdema, and minute vesiculation of the surface, and itching.
+ + + + = redness, marked œdema, and vesiculation of the surface and itching.

neck, due to the wearing of dyed fur collars, were tested. In each case the rôle of the collar in the production of the dermatitis had been proved on several occasions by its disappearance when the fur ceased to be worn, and its subsequent reappearance on the reapplication of the fur to the neck. Three tests were made in each of the three cases, using the

patient's own fur, the brown lambskin and undyed lambskin. Portions of the furs were clipped off the skins, and three applications were made with each fur, a sample of each fur being applied dry, moistened with distilled water, and moistened with saline. The tests were removed and read at the end of 24 hours, and again at the end of a further 24 hours. The results are given in Table II.

The positive reactions to a single application of the dyed furs persisted for 10 to 15 days, passing

TABLE III.

Results of tests with brown lambskin fur in 15 patients suffering from various types of skin disease. The fur was applied dry, moistened with distilled water, and with 0·9 per cent. saline. Each patient was retested after an interval of 16 days.

Disease.	Cases.	First test.		Second test.	
		Pos.	Neg.	Pos.	Neg.
Dermatitis venenata ..	2	0	2	0	2
Dermatitis..	3	0	3	0	3
Dermatitis artefacta ..	2	0	2	0	2
Lupus	1	0	1	0	1
Sycosis	1	0	1	0	1
Impetigo	1	0	1	0	1
Asthma and prurigo ..	1	0	1	0	1
Ulcus cruris	4	0	4	1	3

Pos. = positives; Neg. = negatives.

through a dry scaly stage before the skin resumed its normal appearance.

It is evident from these results that the irritant properties of the dyed lambskin are due to one or more of the constituents used in the dyeing process. It has also been shown that moistening the fur with either distilled water or saline solution increases its irritant properties, although it is to be noted that preliminary moistening is not necessary for the production of a positive reaction. In all cases the patient's own fur and the dyed brown lambskin fur caused some staining of the skin, even when applied in the dry state, and this staining was due to some of the dye becoming dissolved either in the moistening fluids or in the perspiration or oily secretion of the skin. Case 2 had a coarse, greasy skin, and in her the dry applications caused more staining than in Cases 1 and 3. The fact that the reactions to the dry furs were less marked than those to the moist preparations

suggests that the concentration of dissolved dye bears a direct relationship to the intensity of the reaction which it produces.

To control these observations, the brown lambskin fur was applied dry, moistened with distilled water, and with saline, to the skin of 15 patients suffering from various types of skin diseases. The patients were retested with similar applications after an interval of 16 days to verify the results of the first tests and to ascertain whether it was possible for a skin which had shown a negative reaction to the first application to develop an altered reaction as a result of this

FIG. 1.

This patient's skin was greasy, and marked staining of the skin with the dye from a brown lambskin fur applied dry (left) and moistened (right) has occurred. The dry preparation has caused almost as much staining as the moist. No reaction followed either application.

application, so that a positive reaction would be given to subsequent tests. The results are given in Table III.

The fur did not produce a positive reaction in these patients when applied for the first time. In most of these cases all the fur applications produced some degree of staining of the skin, and in several the skin was excessively greasy. This staining is well shown in Fig. 1. It may therefore be concluded that normal skins do not tend to react to the dye dissolved from the brown lambskin fur, even when this substance is in prolonged contact with the skin, either in watery or oily solution. One case, however, developed a positive reaction to all three applications of the fur on testing a second time, although the results of the first test had been negative. This patient's skin apparently developed a susceptibility towards some substance in the fur, and presumably the altered

reaction which occurred had been brought about as a result of the first application.

These preliminary experiments show that the skin of patients who are the subjects of fur dermatitis reacts differently from that of normal individuals to the application of a dyed fur which possesses irritant properties, even when the conditions favourable to the production of a dermatitis reaction are equal in both types of individual. They also show that the skin of a patient suffering from dermatitis, due to some other cause, is not specially apt to react to dyed fur, suggesting that the constituent in the dyed fur responsible for the reaction in susceptible cases is not a universal irritant for eczematous individuals. A specific capacity on the part of the skin to react with such substances appears to be a necessary factor before any reaction takes place. This capacity bears no relationship to the oiliness or moisture of the skin, as shown by the negative reactions obtained in normal individuals in which such conditions were present to a marked degree. The experiments further demonstrate that it is apparently possible for a previously normal skin to develop a sensitivity towards dyed fur as a result of one application of the fur.

EXPERIMENTS WITH GREY LAMBSKIN.

On the basis of these preliminary experiments, a further series of experiments was carried out to test the irritant properties of a grey dyed lambskin, which had been responsible for a few cases of dermatitis. This fur had undergone treatment with solutions of proprietary substances, which will be referred to as A, B, C, and D. Substance A, which belonged to that class of phenylenediamine compounds known as ursols, and which contained paraphenylenediamine, but not metaphenylenediamine, gave a purplish-grey solution when allowed to oxidise in air for several days, and when mixed with hydrogen peroxide developed a medium-grey colour. Solutions of the other substances were colourless. In the dyeing process hydrogen peroxide was used to develop the required grey colour.

Contact tests were performed with this fur in 50 patients suffering from various types of skin lesions, but with no history of previous fur dermatitis. The fur was applied moistened with distilled water,

since in the preliminary experiments with the brown lambskin fur it had been shown that in fur-sensitive individuals a more intense reaction was given when the fur in question was applied moist. Each individual in the series was retested with the fur after an interval of 16 days had elapsed from the time of the first test. The results are shown in Table IV.

TABLE IV.

Results of contact tests with moistened dyed grey lambskin in 50 patients suffering from various skin lesions. The tests were repeated after an interval of 16 days.

Disease.	Cases.	First test.		Second test.	
		Pos.	Neg.	Pos.	Neg.
Dermatitis	22	0	22	0	22
Psoriasis	7	0	7	0	7
Ulcus cruris	4	0	4	0	4
Nævus	3	0	3	0	3
Lupus vulgaris	3	0	3	0	3
,, erythematosus ..	2	0	2	0	2
Impetigo contagiosa ..	2	0	2	0	2
Sycosis	2	0	2	0	2
Prurigo	2	0	2	0	2
Xeroderma	1	0	1	0	1
Lichen planus	1	0	1	0	1
D. herpetiformis	1	0	1	0	1
Total	50	0	50	0	50

The results show that the fur does not contain any substance which has universally toxic properties in the concentrations obtained in the experiment. Again, staining of the skin by the fur was frequently in evidence.

Twelve patients known to have suffered previously from fur dermatitis were then tested with moist applications of the grey dyed lambskin fur. The clinical histories of those patients strongly suggested that the dermatitis from which they had suffered was due to the wearing of dyed fur; the dermatitis persisted until the fur ceased to be worn, and eczema tests with the fur afforded absolute proof of the causal relationship of the fur to the dermatitis. In some cases other types of dyed fur, which were substituted for the originally offending article, also caused dermatitis, while in others a dyed fur had been found which could be worn with impunity.

Each of these patients gave a positive reaction to the test application of the grey lambskin. In four

cases the positive reaction was not evident until 24 hours after the test applications had been removed —i.e., 48 hours after their first application. The reaction was intense in every case, and consisted of redness, œdema, and minute vesiculation, and was accompanied by a considerable degree of itching. From the clinical histories of these patients it will be seen that, although the patient had suffered from dermatitis produced by a fur collar, it had been subsequently possible to obtain and to wear a dyed fur which did not produce any harmful results. This being so, the results of the tests with the grey fur might well have been negative.

The positive results obtained in these cases, when compared with the uniformly negative results found in other types of skin disease, demonstrate the existence of a special reactivity or sensitivity on the part of the skin in the former group. They also show that patients who have shown this type of sensitivity to one particular dyed fur are extremely liable to react similarly to other types of dyed furs.

Since several chemical substances had been employed in the preparation of this fur, these were tested out separately on fur dermatitis Cases 1, 2, 4, 9, and 13, and also on 50 patients suffering from various types of skin disease. The following test solutions were used :—

Solution I. = 0·5 per cent. aqueous solution of "A."
" II. = 0·5 " " " "B."
" III. = 0·5 " " " "C."
" IV. = 0·5 " " " "D."

The results in fur dermatitis cases are given in Table V.

TABLE V.

Results of contact tests with solutions I. to IV. applied for 24 hours in fur dermatitis cases.

Case.	Solution.			
	1	2	3	4
1 ..	++++	—	—	—
2 ..	++++	—	—	—
4 ..	++++	—	—	—
9 ..	++++	—	—	—
10 ..	++++	—	—	—

Forty-nine of the 50 cases suffering from skin disease, other than fur dermatitis, gave a negative

reaction to all four test solutions on the first application, and also on a second application performed after an interval of 16 days. One case gave a strong positive reaction to solution 1 (0·5 per cent. "A"), while the reactions to solutions 2, 3, and 4 were negative. This patient was suffering from a leg ulcer, and had never worn dyed fur. When tested with the grey dyed lambskin she gave a strongly positive reaction.

The above results definitely prove that the potential irritant properties possessed by the grey lambskin fur are due exclusively to the presence therein of "A," or of some decomposition or oxidation product of this substance which had developed in the 24 hours during which it was in contact with the skin. The essential part played by a specific sensitivity of the skin in the production of dermatitis has again been demonstrated, and the results obtained with the fur itself have been confirmed and more accurately defined. The specific sensitivity of the skin which is associated with the dermatitis reaction is not possessed to any extent by normal skins.

In all, 100 cases of various types of skin disease (other than fur dermatitis cases) were tested either with the grey fur or with "A," and of these only one displayed any evidence of sensitivity, whereas a positive reaction was given by all fur dermatitis cases tested. Such a reaction must, therefore, be considered as abnormal, and must depend on some defect in the skin itself.

In order to ascertain whether it was unaltered "A" or its oxidation products which were reacting with the abnormal skin, a 24-hour old mixture of 0·5 per cent. "A" solution and hydrogen peroxide was tested on fur dermatitis Cases 1, 2, 4, 9, and 10. The results are given in Table VI.

TABLE VI.

Results of contact tests with 0·5 per cent. solution of compound "A" mixed with hydrogen peroxide for 24 hours.

Case	1	2	4	9	10
0·5 per cent "A" solution +hydrogen peroxide	—	—	++	—	++

These observations show that the positive results obtained with the fur itself and with freshly prepared 0·5 per cent. "A" solution were due to the skin

reacting either with unchanged "A" or with its early oxidation products, and that the more completely substance "A" was oxidised the less was the sensitised skin likely to react with it.

While the marked contrast between the number of positive reactions obtained in fur dermatitis cases and in non-fur dermatitis cases is strong evidence that, in the former group, the skin is hypersensitive to substance "A," it gives no indication of the degree of this hypersensitivity. The following experiments were performed in order to gain an approximate quantitative estimate of this factor. Fifty patients suffering from various types of skin disease who had previously given a negative reaction either to 0·5 per cent. "A" or to the grey lambskin fur were tested with 1 per cent., 5 per cent., and 10 per cent. solutions of "A." No positive reactions were obtained with the 1 per cent. solution, negative reactions were given by 48 patients to 5 per cent. and 10 per cent. solutions, and two cases reacted to 5 per cent. and 10 per cent. solutions. Fur dermatitis Cases 1, 4, 5, 10, 11, and 12, and the case of leg ulcer, which had given a positive reaction to 0·5 per cent. "A" and to the grey fur (Case 13) were tested with 0·5 per cent., 0·1 per cent., 0·05 per cent., and 0·005 per cent. solutions of "A" and with the grey fur. The results are shown in Table VII.

TABLE VII.

Results of contact tests with grey lambskin fur, 0·5 per cent., 0·1 per cent., 0·05 per cent., and 0·005 per cent. solutions of "A" in fur dermatitis and "A" sensitive cases.

Cases.	Fur.	Solutions of "A" (per cent.).			
		0·5	0·1	0·05	0·005
1	++++	++++	+++	+++	—
4	++++	++++	+++	+++	+
5	++++	++++	+++	—	—
10	++++	++++	+++	—	—
11	++++	++++	+++	—	—
12	++++	++++	—	—	—
13	++++	++++	++++	++++	++++

These experiments show that the difference in sensitivity to dyed fur between a skin which is intolerant to it and a normal skin is of considerable magnitude. Thus Cases 4 and 13 react to at least 1/2000 of the concentration of "A" to which a

normal skin is tolerant. Case 4, Cases 5, 10, and 11, and Case 12 react, respectively, to 1/200, 1/100, and 1/20 of the same concentration. The position might be regarded from the opposite standpoint and Cases 4 and 13 considered as being at least 2000 times, Case 4 200 times, Cases 5, 10, and 11 100 times, and Case 12 20 times as sensitive to "A," as the normal skin. Moreover, it is possible that the normal skin would tolerate concentrations of "A" higher than 1 : 10, in which case the sensitivity of the reacting skins would be correspondingly greater.

When the positive reactions obtained with varying dilutions of "A" are compared with those obtained with the ursol-dyed fur, it is possible to gain some idea of the concentration of "A," which is presumably dissolved out of the fur and brought into contact with the skin to give rise to the positive skin reaction produced by the fur. In Cases 1 and 2 this must have been between 0·05 per cent. and 0·005 per cent.; in Cases 5, 10, and 11 between 0·05 per cent. and 0·1 per cent.; and in Case 12 between 0·5 per cent. and 0·1 per cent. Case 12 further demonstrates that it is possible for a solution of "A," having a concentration of at least between 0·1 per cent. and 0·5 per cent., to be formed from the grey fur when this becomes moist.

An interesting and important point, conclusively shown by the results of the tests with different concentrations of "A," is that the sensitivity of the skin in fur-sensitive cases shows wide individual variations. The results also show, although less definitely, that the intensity of the reaction in individual cases varies directly with the concentration of "A." It seems probable, however, that the range of concentrations throughout which graded reactions occur is small, and that, once a certain concentration is reached, the intensity of the reaction obtained is maximal.

THE EFFECTS OF CLEANING.

The substance in the grey fur which took part in the production of the reaction exhibited by abnormal skins when in contact with the fur having been identified as substance "A" or an early oxidation product of this substance, attempts were made to ascertain whether it could be removed by special methods. For this purpose seven samples of lambskin fur dyed with "A," but subjected to different cleaning

processes, were tested on eight fur-sensitive cases, along with the original grey lambskin used in the previous experiments. The cleansing processes consisted in washing in water or benzene for varying periods, brushing, or drumming with sand. It was not possible to test out all eight samples in each case, while in some individual cases the same sample was tested on several occasions. The results are given in Table VIII.

TABLE VIII.

Results of contact tests with original "A" dyed grey fur (fur 1) and with seven grey furs dyed with compound "A," and subjected to various cleansing processes, in fur-sensitive cases.

Case.	Fur.				Sample.			
	1	2	3	4	5	6	7	8
1	+ + + +	0	0	0	0	−	+ + +	+ + +
2	+ + + +	0	0	0	0	−	+ + +	+ + +
4	+ + + +	+ +	+ +	+ +	+ +	−	+ + +	+ + +
5	+ + + +	+ +	0	+ +	0	−	+ + +	+ + + +
*6	+ + + +	+ +	+ +	+ +	+ +	−	+ +	+ + + +
7	+ + + +	+ +	+ +	+ +	−	−	+ +	+ + +
8	+ + + +	+ + +	+ + +	+ + +	+ +	−	+ + +	+ + + +

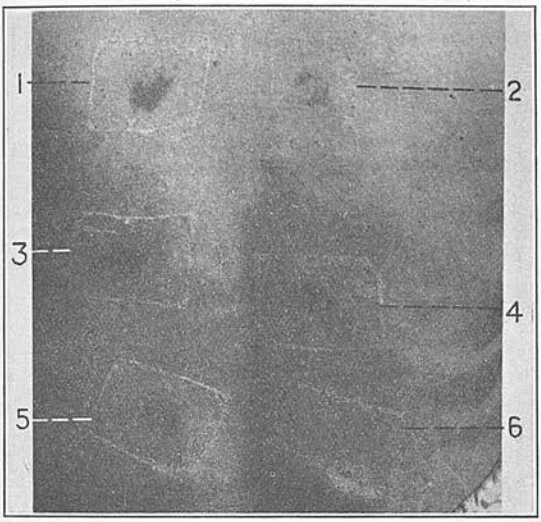
* Reactions to samples 1–6 shown in figure (2). 0 = No test performed.

This Table shows that with adequate cleaning methods it is possible to reduce considerably, and perhaps to eliminate altogether, the substance in the dyed fur which causes the hypersensitive skin to give a dermatitis reaction. A comparison with the reactions given by Case 4 in Tables VI. and VII. shows that any solution of "A" derived from fur 6 when in contact with the skin must have been of a lower concentration than 0·005 per cent., since Case 4 was capable of reacting to this concentration, whereas she gave no reaction to fur 6.

In this investigation the reactions given by Case 4 were of special interest. This patient was first tested with furs 1, 3, and 6. No reaction was obtained with fur 6, while a well-marked reaction occurred with fur 1 and a papulo-erythematous vesicular reaction occurred with fur 3. The reaction to fur 1 was still visible at the end of five days as a dry, pink, scaly area, but that to fur 3 had almost faded. At this time fur 1 was reapplied, together with

furs 2, 4, and 5 ; 24 hours later the tests were read and positive results (Fig. 3) were found in each case. During the following few days a flare-up of the subsiding positive reactions to the first application of fur 1 and of fur 3 occurred. This exacerbation gradually subsided, along with the reactions to the second application of fur 1 and of furs 2, 4, and 5

FIG. 2.



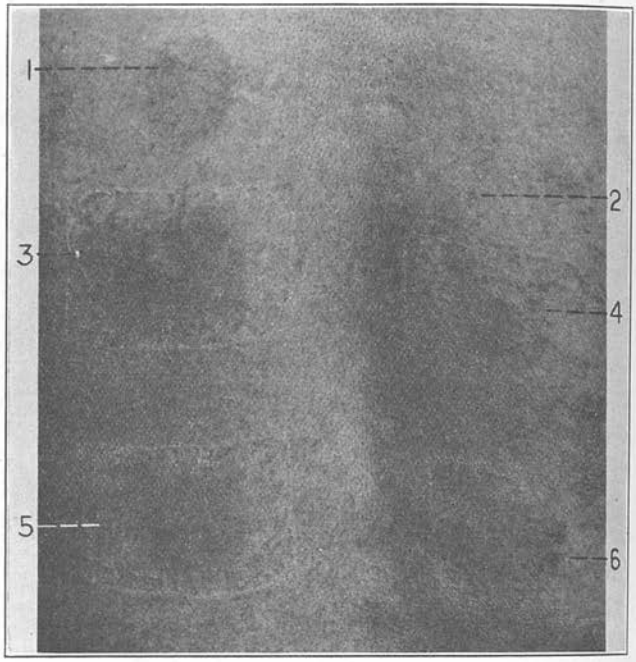
Case 6, Table VIII., tested with fur samples 1–6.

				After application of test—
1 =	Reaction to fur	3	.....	36 hours.
2 =	,, ,, ,,	2	.....	36 ,,
3 =	,, ,, ,,	1	.....	36 ,,
4 =	,, ,, ,,	4	.....	36 ,,
5 =	,, ,, ,,	5	.....	36 ,,
6 =	,, ,, ,,	6	.....	36 ,,

during the succeeding 37 days. Twelve days after the second application of fur 1, and at a time when the reactions to this application and to furs 2, 4, and 5, and also the exacerbation of the reactions to the first application of furs 1 and 3, were subsiding, a third application of fur 1 was made. A well-marked erythemato-vesicular reaction followed

(Fig. 4), but, contrary to expectations based on the experience with fur 3, there was no general flare-up of the fading reactions to furs 1, 2, 3, 4, and 5. Fifty days from the date of the first test applications the skin of the back had resumed its normal appearance. Fur 3 was again applied

FIG. 3.



Reactions given by Case 4 to samples 1, 2, 3, 4, and 5.

		After application of test—
1 =	Reaction to fur 1	5 days.
2 =	,, ,, ,, 3	5 days.
3 =	,, ,, ,, 1	30 hours.
4 =	,, ,, ,, 2	30 ,,
5 =	,, ,, ,, 4	30 ,,
6 =	,, ,, ,, 5	30 ,,

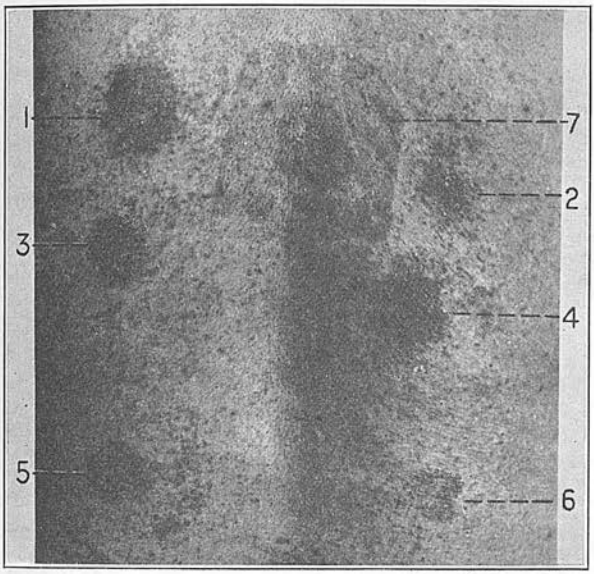
at this time, and a positive result was obtained at the site of application only (Fig. 5). The interesting feature of these results is the flare-up

of subsiding positive reactions on one occasion, which was coincident with the application of fresh tests to adjacent areas. This point will be discussed later.

ATTEMPTS TO TRANSFER SKIN SENSITIVITY TO NORMAL SKINS.

Serum was obtained from Cases 4 and 13, both of whom had shown the greatest skin hypersensitivity

FIG. 4.



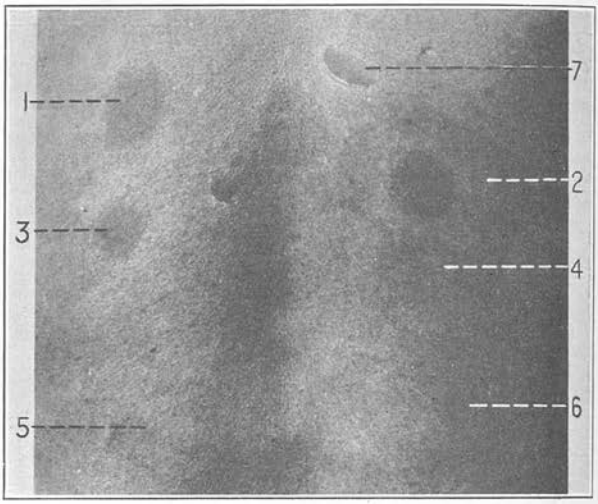
Reaction given by Case 4 to samples 1, 2, 3, 4, and 5.

			After application of test—
1 =	Persisting reaction to fur	1	17 days.
2 =	" " " "	3	17 "
3 =	" " " "	1	12 "
4 =	" " " "	2	12 "
5 =	" " " "	4	12 "
6 =	" " " "	5	12 "
7 =	Reaction to "	1	30 hours.

to "A," and 0·2 c.cm. of each sample was injected intradermally into four areas of the skin of the forearm in four individuals who had given a negative

reaction to contact tests with 10 per cent. "A." After four hours the injected areas were tested with 0·5 per cent. "A" applied to a scratch and also as a contact test. (Method of Prausnitz and Küstner.) Control scratch and contact tests were made on adjacent uninjected skin. No positive reactions were obtained with any of the tests. Both forms of test were repeated 24 hours later, but again the results were negative.

FIG. 5.



Reactions given by Case 4 to samples 1, 2, 3, 4, and 5.

1, 3, 4, 5, 6, 7 represent the corresponding sites in Figs. 2 and 3 50 days after the first tests were applied. The reactions have completely faded, leaving only a faint pigmentation of the skin.

2 = Reaction to fur 3, 36 hours after the application of the test.

SUMMARY OF THE FOREGOING EXPERIMENTAL RESULTS.

(1) The potential irritant properties of a dyed fur are due to some substance used in the dyeing process, and not to mechanical irritation by the fur.

(2) Normal skins do not react to a dyed fur even under conditions in which the fur in question would produce dermatitis in a fur dermatitis patient.

(3) The dermatitis is due to a hypersensitive skin reacting with some substance in the dyed fur in concentrations with which normal skins do not react, and, therefore, this reaction may be termed allergic.

(4) The substance in the fur investigated with which the allergic skin reacts is "A," or an early oxidation product of this compound.

(5) Fur dermatitis cases react to concentrations of "A," varying from 0·005 per cent. to 0·5 per cent., and in these cases there is considerable individual variation with regard to the concentration necessary to produce dermatitis.

(6) Most normal skins fail to react with a 10 per cent. solution of "A" although some were found to react with a 5 per cent. solution.

(7) The degree of sensitivity of the skin to dyed fur found to exist in fur dermatitis cases ranged from at least 200 times to 2000 times that found in non-fur dermatitis cases.

(8) It is possible for at least a 0·5 per cent. solution of "A," or its oxidation products, to be formed in the fluid secretions of the skin when in contact with the dyed fur. This type and concentration of solution does not produce dermatitis in non-fur dermatitis cases.

(9) Dermatitis resulting from a single application of a minute concentration of a chemical towards which the skin is allergic may persist for several weeks.

Comment.

Two conclusions may justifiably be drawn from the results obtained in the foregoing experiments. The first is that "A" is the only factor present in the fur which takes any part in the production of dermatitis. The second, and the more important from the point of view of its general application, is that the dermatitis depends on an allergic state of the skin, and that it is idiosyncratic in nature. The term allergy is here used in its wider sense, according to the definition of Bloch, which is as follows :—

"Allergy is that state which has as its basis the property of certain groups of cells (organs) of the living organism to react in a specific manner when brought in contact with a substance which is, as far as is known, foreign to the organ or cells; the characteristic of this specific pathologic

process lies in the fact that it is caused by the reaction of this exogenous substance with its specific cellular fixed antibody. The basis and essence of allergy is the ability of the living cell to react with the production of specific antibodies to the stimulus of foreign substances, which are therefore called antigens, as well as the fact that the contact of the antigen with its specific cellular fixed antibody causes a disturbance of cellular life which usually results in an inflammatory reaction."

The term idiosyncrasy means that the reactions caused by the exogenous substances in question occur only in a small percentage of individuals. The reason for idiosyncrasy is therefore the presence of the allergic state in those individuals who are idiosyncratic. As a result of allergy, idiosyncratic individuals react to substances which produce no effect in normal individuals. Such substances are, therefore, irritant and toxic for the former class, but perfectly harmless for the latter. This toxic effect, however, is not proportional to the dose or concentration of the substance coming in contact with the cells, but is entirely dependent on the state of these cells. The substance only precipitates a reaction when the cells are in a state of allergy. Such a reaction is not due to the pharmacological action of the substance, since this would be approximately the same in all individuals, and would be proportional to the amount of the substance acting on the cells. It has been shown that allergic phenomena, which were originally considered to be brought about only by antigens of a protein nature, can be caused equally well by crystalline substances.

The results of the tests show conclusively that positive reactions occur only in idiosyncratic individuals, and that they are due to the allergic state of the skin towards "A." In this class of individual the intensity of the reaction bears no constant relationship to the concentration of "A" applied to the skin. In one case a concentration of 0·5 per cent. may be required, while in another 0·005 per cent. will suffice. The uniformly negative results obtained with 0·5 per cent. "A," and the large percentage of negative results obtained with 10 per cent. "A," in individuals who had not suffered from fur dermatitis, show that even in these strengths the pharmacological action of compound "A" cannot be described as irritant. Nevertheless, throughout the range 0·5 per cent. to 10 per cent. several positive reactions were obtained, and these individuals may be considered

idiosyncratic in the same way as those sensitive to lower concentrations, but in the former case the allergy is of a less delicate or sensitive nature. It is possible that with concentrations higher than 10 per cent. compound " A " would display universal irritant properties, which would then depend on the pharmacological action of the drug, be proportional to the dose, and independent of the state of the skin in individual cases. When it is considered that reactions were obtained with concentrations from 1/200 to 1/2000, which was tolerated by normal individuals, it is obvious that some factor other than the direct action of " A " must be mainly responsible for the reaction. This factor can only be the altered state of reactivity of the skin cells, or, in other words, allergy. Apart from the evidence derived from a comparison of the active concentrations in reacting individuals and the concentrations tolerated by individuals, the behaviour of the positive reactions which were obtained denotes their allergic nature. The latent period observed between the removal of the test application and the appearance of a positive result, and the tendency for positive reactions to flare-up as a result of subsequent applications of " A," are both typical of allergic phenomena.

The results obtained by Burgess and Usher[6] are comparable to the more general findings in the present investigation. They found that of 200 normal persons tested with a face lotion which had been associated with dermatitis 0·5 per cent. gave a positive reaction, while a reaction was obtained in all patients who had suffered from the dermatitis. They record the interesting observation that in several of these dermatitis patients a positive reaction could only be obtained at the site of the healed dermatitis, so that in those cases the allergy was localised to certain skin areas. No example of regional allergy was found in the present investigation, and in every case the allergic phenomena were elicited over wide areas of skin. Beyond demonstrating the idiosyncratic and allergic nature of the disease, and proving that only one constituent of the lotion was capable of producing it, Burgess and Usher performed no experiments to demonstrate the quantitative nature of the allergy.

The fact that in the present experiments positive reactions were only obtained when " A " was applied to the intact skin, and that uniformly negative

results were obtained by the scratch method, points to the allergy being localised in the epidermis. It also points to the necessity of carrying out both types of test when an allergic condition of the skin is suspected.

Attempts to demonstrate the presence of free antibodies in the serum, by the passive transference method of Prausnitz and Küstner, and so to prove the antibody-antigen nature of the positive reactions obtained in the skin, failed in three cases. Successful results, although frequent in urticaria with this method, have only rarely been obtained in cases of dermatitis. Biberstein has, however, actually demonstrated the Prausnitz-Küstner reaction in ursal dermatitis. A negative Prausnitz-Küstner reaction merely suggests that demonstrable antibodies are not present in the serum, and does not exclude the probable antigen-antibody nature of the allergic reaction of the skin cells.

The facts observed with compound " A " therefore show that any skin reaction which occurs with this substance in low concentration is due to the allergic conditions of the skin, and not to the primary irritant action of " A." There are ample opportunities for " A " present in a fur to dissolve in the secretions of the skin when the fur is brought into contact with the skin, and it is probable that the allergic skin only reacts to " A " when in solution.

The latent period observed clinically between the first time of wearing a fur and the development of dermatitis, which may amount to a few weeks, may be due to several factors. When short, it is probably a delayed reaction to the first contact with the fur, similar to that noted in the reaction to skin tests. When of longer duration, it may either be due to an initially non-allergic skin developing allergy, as has been shown to be possible in the preliminary experiments, or to the fact that in an allergic person no direct contact between the skin and the dyed fur has occurred during the first two or three occasions on which the fur has been worn.

The results obtained in the foregoing experiments suggest that similar processes are responsible for the production of dermatitis by furs dyed with other amines, diamines, aminophenols, diphenols, and their substitutes. No definite comparisons are justifiable, however, and any fur which has been associated with

dermatitis must be subjected to similar biological analysis before a definite statement can be made regarding the nature and cause of the dermatitis. Chemical analysis of the fur yields no information as to its possible biological effects. It is a well-known clinical fact, and one which is illustrated by the case-histories of the fur-sensitive cases here reported, that an individual may suffer from dermatitis when wearing one dyed fur, but not another. The reason for this may be that the pre-existing or subsequently developed allergic state of the patient's skin is specific for one amine body, or that the fur which is not associated with dermatitis contains no available antigen.

It has already been pointed out that the experiments with "A" show that this substance is not, strictly speaking, an irritant, and that when signs of inflammation follow its application to the skin in an idiosyncratic person this is entirely due to the skin being in a highly intolerant or allergic state. From the series of tests carried out with "A" dyed fur and with "A" on unselected cases of skin diseases, it appears that a very small proportion of individuals exhibit an idiosyncracy to "A," and when the number of cases of fur dermatitis is compared with the total number of fur-wearers this proportion is infinitely less. Incidentally, it has been shown that the existence of various types of skin disease, other than fur dermatitis, are not associated with an unduly high proportion of individuals sensitive to "A." When several substances to which individuals are known to have shown an idiosyncrasy are compared, it is found that the number of idiosyncratic individuals varies greatly according to the substance. Thus Bloch[7] quotes experiments to show that 80 per cent. of all individuals are sensitive to ascaris, 60 per cent. to poison ivy, and 45 per cent. to orthoform. Moreover, by using concentrated extracts of primula, he has been able to produce an allergic state in the skin of 100 per cent. of persons, thus overcoming the individual constitutional factor (idiosyncrasy), and converting an idiosyncrasy into a pure allergy. These facts would have to be born in mind when assessing the extent to which any compound in a dyed fur was capable of participating in or precipitating allergic reactions of the skin.

They also have an important medico-legal bearing. First, the fundamental rôle played by allergy in

such cases, and, secondly, the proportion of normal to idiosyncratic individuals ought to be considered when compensation claims arise.

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